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CULTIVATION EXPERIMENTS ON THE GLOBOID BODIES OF POLIOMYELITIS.*

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The microbic cause of poliomyelitis is a subject still in the foreground of interest with respect to the etiology of that disease. The present paper deals with the conditions surrounding the cultivation of the globoid bodies described by Flexner and Noguchi (1) from poliomyelitic tissues and is intended as a contribution to the technique of the method.

The technique of the cultivation is a complex and difficult procedure, even more difficult, perhaps, than the isolation of *Treponema pallidum*. With respect to the treponema, the experimenter is assisted by the fact that it is comparatively large, possesses clear-cut morphological characteristics, and is motile—properties which make its detection relatively easy.

The globoid bodies, on the other hand, are extremely minute and non-motile. They are not readily distinguished from tiny particles of detritus in the early generations of scantily growing cultures. Hence weeks, even a month or more, may elapse between the initial inoculations of the culture medium and the definite determination of successful cultivation of the organism; and it may happen that the growth then observed may fail to develop on transplantation, so that the opportunity for its identification may be lost. The difficulties surrounding the method of cultivation have resulted in the confirmation of Flexner and Noguchi's work by only a few bacteriologists, the larger part of those who have attempted to repeat it failing in their efforts.

The present study was undertaken in the hope of modifying the

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method by rendering it simpler and more certain of success. The original procedure is not only uncertain in its ultimate results, but also in the number of cultures yielded by a given lot of tubes inoculated with poliomyelitic tissue. Sometimes of 30 to 50 tubes thus inoculated, one or two only would yield cultures, the others remaining without demonstrable growth. Where the conditions all appear to be so similar, it would seem that a higher percentage of cultures should be obtained. Obviously the conditions within the tubes varied in an unaccountable manner; and it became also the purpose of this study to eliminate the factor of variation and thus to increase the proportion of cultures obtainable from a certain number of inoculated tubes.

Hitherto, the globoid bodies have been cultivated from the central nervous organs, which are the seat of the profounder effects of the disease. Now, according to present conceptions of the pathology of poliomyelitis, the disease partakes often of the nature of a general or systemic infection. Indeed, the non-paralytic or abortive cases, so called, pursue a course unattended by paralysis or even by marked symptomatic involvement of the central nervous system. Hence it became desirable to study the general viscera by culture methods.

The source of the tissues submitted to cultivation was *rhesus* or *cynomolgus* monkeys in which the inoculation form of the disease had been produced. The usual procedure was to permit the paralysis to develop to the point at which the inoculated animals became prostrate; they were then etherized. In certain instances animals were used which had been prostrate for several days or which had died some hours earlier. A comparison of the cultures from the two classes of animals—those recently paralyzed and still in good physical condition with those which have been *in extremis* for some time or have actually succumbed to the disease a number of hours before autopsy—is illuminating as regards the number and kind of cultures obtained.

The virus or emulsion of the nervous organs employed for inoculation was either an active virus which has been transmitted in the laboratories of The Rockefeller Institute for several years, or the less active M.A. virus (2), which has also passed through many monkeys. The mode of inoculation was usually intracerebral, but

sometimes it was by other routes—the nose and blood. A brief summary of the clinical data of the infected monkeys is given in Table I.

We propose to give the details of the technique of the successful cultivations of the globoid bodies, which will be followed by a description of the results obtained by the method, including both success and failure, and the probable causes of each.

TABLE I.

Summary of Clinical Data of Monkeys Infected with Poliomyelitic Virus.

Serial No.	Source of virus.	Amount inoculated.	Route of inoculation.	Duration of symptoms.	Duration of prostration.	Length of illness.	Pathologic appearance.
				days	*days*	*days*	
1	Fresh mixed.	50.0 cc.	Intravenous.*	1½	½	7	Typical.
2	Glycerolated mixed.	2.0cc suspension	Intracerebral.	4	1	13	"
3	Fresh mixed.		Nasal.	9	6	18	"
4	" "	0.2 cc. filtrate.	Intracerebral.	3	1	10	"
5	" "	50.0 cc.	Intravenous.*	2	0	7	"
6	" "	0.1 "	Intracerebral.	1½	¼	10	"
7	" "	0.1 "	"	1	0	11	"
8	" "		Nasal.	3	1	11	"
9	" "	0.1 cc.	Intracerebral.	2	1	7	"
10	" "	0.2 "	"	8	1	13	"†
11	" "	0.2 "	"	7	3	15	"†
12	M.A.	1.0 "	"	5	1½	9	"
13	Fresh mixed.		Nasal.	4	2	12	"
14	" "		"	2	½	10	"
15	" "	2.0 cc.	Intracerebral.	2	1	6	"
16	" "		"	2	½	6	"
17	" "		Nasal.	3	1	7	"
18	M.A. cultivated.	2.0 cc. mass culture.	Intracerebral.	5	0	12	"

* Intravenous inoculation of centrifuged emulsion following the injection of horse serum intraspinally.

† These animals had been dead 7 and 3 hours respectively when autopsied.

Cultivation Technique.

The methods used for the cultivation of the globoid bodies from the tissues of monkeys are essentially the same as those originally

described by Flexner and Noguchi (1). Certain variations of the technique will, however, be described. In part, the technique is a repetition of previously reported work, but is given as a convenient summary.

Test-tubes, 1.5 by 20 cm., should be used for all experiments, for the narrower tubes are much more likely to become contaminated. They should be plugged with cotton and placed in a surgical package containing 25 to 30 tubes, which should then be sterilized and not opened until the set of tubes is to be used.

Fresh sterile tissue is added to each tube. A healthy, fat, adult rabbit is chosen, one that has not even a scratch or other detectable defect upon any part of its body. The animal, which must be given no food for 24 hours, is lightly etherized and bled to death from the heart by means of a sterile needle and a large bulb pipette. All hair is then removed from the abdomen and anterior thoracic walls of the animal's body by sodium sulfide solution, and the skin is prepared with the usual surgical procedures, soap and water, bichloride solution, alcohol, and finally iodine. Instruments for obtaining the kidneys should not be boiled, but required instruments are made into a set and sterilized in a glass container in the hot air sterilizer. One set is used to open the skin, another set to open the peritoneal cavity, and a fresh pair of scissors and forceps are used to remove the kidneys. All procedures should be carried out in a dust-proof room, or under a hood, the walls of which have been wiped down with bichloride solution.

After removal to a large sterile Petri dish, the capsule is stripped from the kidney, each kidney is cut into 20 to 25 pieces, and one bit of tissue placed in each sterile test-tube. This procedure must also be carried out in a dust-proof room. While removing the capsule of the kidney and cutting it into pieces, the assistant should hold the cover of the Petri dish directly over the field of operation in order to avoid dust contamination.

The poliomyelitic material which is added to each tube consists of a bit of tissue of about the same size as the bit of rabbit kidney tissue. The infected monkey should not be allowed to die, nor, in fact, to become prostrate, but should be etherized at the height of the active symptoms. After etherization, the animal should be bled, and the visceral tissues, liver, spleen, kidney, thymus, etc., removed with exactly the same technique as was used in obtaining sterile rabbit kidney. It is important to use a separate pair of forceps and scissors for each organ.

The technique for obtaining uncontaminated poliomyelitic brain tissue is as follows: The calvarium is removed with aseptic precautions, great care being taken not to injure the dura. The dura over the hemisphere opposite the one that had been injected is then seared with a red hot knife blade, and the dura is opened with sterile instruments. A portion of the cerebrum is removed with a separate set of instruments and placed in a sterile Petri dish. This material is at once taken to a dust-proof room, when it may be cut into suitable pieces and placed immediately in the tissue test-tubes.

Methods for removing bits of sterile spinal cord are very unsatisfactory. The following method has proved the most useful. The entire cord is exposed under aseptic precautions, great care being taken not to injure the dura. A portion of the cord, 3 to 4 cm. long, is tightly tied at either end with a sterile thread and the portion removed. This bit of cord with intact dura is placed in bichloride alcohol for 60 seconds, then rapidly passed through five separate washings of sterile salt solution. The dura is wiped dry with sterile gauze, seared with a narrow hot knife blade, and is then opened with sterile instruments and the cord cut into 0.5 cm. segments, each portion being put into a tissue test-tube.

When the test-tubes have been inoculated with rabbit kidney and poliomyelitic material, ascitic fluid should be added, 15 or 18 cc. being sufficient. The ascitic fluid must, of course, have been tested carefully for sterility, both for aerobic and anaerobic organisms. It should have a specific gravity of at least 1,015, be clear, free from bile, not more than 3 months old, and should be stored in the refrigerator. It should not be heated above 50°C. or filtered. Chylous or bloody ascitic fluid is not suitable. *Before adding the ascitic fluid to the inoculated test-tubes, it should be warmed to 45°C. and placed under a vacuum to remove the air.*

Complete anaerobiosis must be obtained if one is to be successful in the cultivation of the globoid bodies. In our first experiments the vacuum jar was used, but the results were disappointing. The vacuum jars were either so poorly ground that they would not hold a vacuum, or, owing to defects in the glass, they cracked or exploded under the tremendous negative pressure. These difficulties were finally met by the use of the hydrogen-nitrogen jar, which utilizes the catalytic action of platinized asbestos upon hydrogen and oxygen, and removes all traces of oxygen from the jar. The details of this apparatus have already been published (3). Subsequent experiments with this jar have given most satisfactory results. The pyrogallic acid-sodium hydroxide indicator has remained unchanged in color for weeks, showing complete anaerobiosis; and the method is so rapid, clean, and simple that it seems to promise a simple satisfactory solution for the problem of anaerobic technique.

The inoculated ascitic fluid-tissue tubes, usually twenty-five to thirty tubes, are placed in the anaerobic jar and incubated for 11 to 12 days. Control tubes, inoculated with brain and other tissues, are incubated in the open air and examined daily in order to determine the presence of contaminating organisms.

After 12 days' incubation, the jar is opened, and 0.2 cc. of the ascitic fluid is removed from the bottom of each tube, without disturb-

ing the bits of brain or kidney tissue. The special pipettes devised by Gates (4) are very useful for all poliomyelitic culture work. The material is transferred directly into freshly prepared tissue tubes. 15 cc. of air-free, warmed ascitic fluid are added to each tube as before, the tubes placed in the anaerobic jar, and at once returned to the incubator. The original tubes may be placed in the refrigerator to be examined at leisure.

The second generation of ascitic fluid-tissue tubes is incubated for 12 days and the anaerobic jar is then opened. As before, immediate transfer is carried out, 0.2 cc. of the ascitic fluid near the bottom of the tube being transferred to freshly prepared tissue tubes. To a second set of tissue tubes is added 0.1 cc. of the material from the bottom of the tubes of the second generation. To Set 1, containing 0.2 cc. of transferred material, is added warmed air-free ascitic fluid as before. To Set 2, containing 0.1 cc. of the material, are added 15 cc. of semisolid medium which has been prepared as follows:

> Veal infusion agar, 2 per cent, with a reaction of +0.5 to phenolphthalein, is melted, boiled, and then rapidly cooled to 50°C. Ascitic fluid, clear, straw-colored, free from bile, with a specific gravity of 1,015 or higher, is warmed to 45°C. and placed under a vacuum to remove the air. 1 part of the melted agar, cooled to 50°C., is added to 2 parts of warmed ascitic fluid, and 15 cc. of the mixture are added to each inoculated test-tube of Set 2.

The two sets of tubes are at once placed in separate anaerobic jars and incubated. The second generation of ascitic fluid tubes from which the transfer was made is placed in the refrigerator and examined on subsequent days for the presence of the globoid bodies.

The anaerobic jar containing the set of semisolid tubes (Lot 2) is incubated for 8 days, and the jar is then opened and the tubes are examined. If any of the tubes contain suspected colonies in the depths of the semisolid medium, these tiny colonies are removed by means of a capillary pipette and examined microscopically. If positive, fresh semisolid tissue tubes are made from each colony, and these tubes are placed in the anaerobic jar and incubated. If none of the tubes show visible colonies, they are all replaced in the anaerobic jar and reincubated for 8 days. If at the end of this reincubation there is no growth in the semisolid tubes, the set is discarded.

The anaerobic jar containing the ascitic fluid tubes of the third

generation, Lot 1, is incubated for 12 days, then opened, and fresh sets of ascitic fluid and semisolid tissue tubes are made exactly as described for the second generation. This process of making ascitic fluid and semisolid tubes is continued for at least five generations, after which time, if no growth has been obtained, all tubes are discarded. Every original tube inoculated is therefore a separate experiment and must be given its own number, and a detailed record must be kept of each subsequent generation of that culture. Each lot of ascitic fluid tubes, after their period of incubation, is kept in the refrigerator, so that one may subsequently return to the earlier generation in case it is necessary to pick up a positive culture that may have died out in subsequent generations.

For example, Culture 6, brain tube No. 1, after growing for two generations in semisolid medium, refused to grow further in this medium, so that it was necessary to return to the ascitic fluid tube, brain tube No. 1, third generation, from which the strain had originally been obtained, and though the growth was very scanty, and the tube had been in the refrigerator for more than a month, nevertheless a positive permanent culture was obtained.

Criteria for the Determination of the Globoid Bodies.

It is necessary to have definite criteria for the determination of the globoid bodies. These have been described in the original publication by Flexner and Noguchi (1) and in later publications and need simply be summarized for convenience.

Morphology and Staining Characteristics.—Morphology and staining characteristics are somewhat variable factors, the variation depending not so much upon the organism as upon the observer, and, though confirmatory, they are insufficient indices in the identification of an organism.

Characteristic Growth in Semisolid Medium.—It is not enough to determine the presence of an organism in the ascitic fluid medium which seems to the observer to possess the characteristic morphology of the globoid bodies. Semisolid medium should be inoculated and observed daily. There should be no apparent growth in the medium for 3 or 4 days. At the end of 72 hours or later, the characteristic minute colonies begin to appear, being definitely established only at the end of 6 to 7 days. In the anaerobic jar, the line of demarcation of growth is about 1 cc. from the surface of the medium. If the semisolid tube has been incubated in the open air, however, only a few colonies will be seen about the tissue in the bottom of the tube and extending upward about 1 cc.

Action upon Carbohydrates.—An additional factor for the identification of the globoid bodies depends upon their inability to attack carbohydrates. Tissue tubes of ascitic dextrose broth are inoculated with a small fragment of a semisolid culture. The broth should be sugar-free veal infusion, +0.2 to +0.3 acid to phenolphthalein. To each 5 cc. tube of broth is added an equal amount of unheated sterile ascitic fluid plus sufficient 10 per cent sterile sugar solution to give a concentration of 0.5 per cent. The hydrogen ion concentration of the medium should be approximately that of blood neutrality; namely, 7.4. Control tubes should be made both of uninoculated tubes and of tubes inoculated with various types of streptococci. The tubes should be incubated in the anaerobic jar for 7 days and the hydrogen ion concentration determined. The globoid bodies will not attack the simple sugar, whereas the control tubes of streptococci will show a marked increase in acidity.

Results Obtained in the Cultivation of the Globoid Bodies.

Material from eighteen poliomyelitic monkeys has been used in the attempted cultivation of the globoid bodies. For the first four experiments, the anaerobic technique used was the vacuum jar, and in each instance the experiment was unsuccessful. For all the later experiments, the platinized asbestos method was used, and far more satisfactory results were obtained.

The material from three of the monkeys was contaminated with a pure culture of streptococcus in the first generation in many or all of the tubes. This contamination was not due to faulty technique, because all control tubes were negative, but was the result of a secondary invading streptococcus which is commonly found in the blood and tissues of animals and human beings who have been prostrate and moribund for hours or days before death finally occurred.

Of the remaining eleven monkeys, the typical globoid bodies were cultivated from seven, and a total of twenty-two separate cultures or strains was obtained. The largest number of strains obtained from a single monkey was six, whereas in two instances only one strain was obtained. Besides the twenty-two strains, eleven other strains were cultivated in the ascitic fluid medium, but I was unable to transfer them to semisolid medium, and they are, therefore, not included in the total number of cultivations. The eleven strains, incompletely isolated, were derived in part from monkeys yielding completely isolated strains. Table II summarizes the successful and partially successful results of the cultivations.

TABLE II.

Successful and Partially Successful Cultivation of the Globoid Bodies.

Serial No.	Generation carried before discarding.	Virus cultivations in semisolid medium.			Virus cultivations in ascitic fluid medium but not transferred to semisolid medium.		
		No. of strains obtained.	Source.	Generation in which culture was obtained.	No. of strains obtained.	Source.	Generation in which culture was obtained.
1	3rd	None.			None.		
2	4th	"			"		
3	12th	"			1	Mesenteric node.	4th
4	9th	"			1	Kidney.	4th
5	10th	1	Brain.	3rd	1	Brain.	4th
6	12th	2	" 1, cord 1.	5th 6th	1	"	4th
7	10th	4	Brain.	4th	None.		
8	11th	3	"	4th	"		
9	3rd	None.			"		
10	2nd*	"			"		
11	1st*	"			"		
12	12th	5	Brain.	1, 3rd 1, 4th	"		
13	8th	6	" 4, spleen 2.	4th	1	Adrenal.	4th
14	7th	None.			3	Brain 2, spleen 1.	3rd
15	4th	"			1	Brain.	3rd
16	4th	"			2	"	3rd
17	1st*	"			None.		
18	4th	1	Brain.	3rd	"		
Total.....		22			11		

* All tubes contaminated with a streptococcus.

Nineteen of the completely isolated strains were obtained from brain substance, one strain was cultivated from the cervical portion of the spinal cord, and two were cultivated from the spleen. Of the eleven incompletely isolated strains, seven were cultivated from the brain, one from the kidney, one from a mesenteric node, and one from the spleen. The shortest period of time between the making of the cultures at autopsy of the monkey and definite establishment of a positive culture was 28 days, and in the third generation. The longest

period required for the definite establishment of a strain in semisolid medium was 54 days, although the organism in this case had been found in the ascitic fluid tube in the third generation on the 30th day.

In no instance was a definitely positive culture found in the first generation of tubes, but usually the organisms were present in sufficient numbers in the second generation to suggest at least that the result would eventually be positive. The globoid bodies are so small and may so readily be confused with detritus, that even a presumptive decision should not be reached unless characteristic forms are found under at least five different microscopic fields. The organisms are so few in number in the second generation that a presumptive test, though possible, is a long and tedious process. It is much simpler and more satisfactory to make subcultures of all tubes of the second generation into the semisolid medium and thus reach a positive conclusion in the succeeding generation by means of the typical growth in colony form.

Occurrence of Streptococci and Other Organisms.

The occurrence of streptococci in certain series of the cultures has been noted. More rarely other microorganisms than streptococci were cultivated instead of or with the globoid bodies. We should consider the sources and endeavor to estimate the significance of these classes of organisms. It is obvious that culture tubes which require so much manipulation will sometimes become contaminated. This condition will account for the miscellaneous bacteria sometimes encountered in the tubes, but probably not for the streptococci which have come to occupy a position of prominence, even if not of importance, with regard to the vexed question of the etiology of poliomyelitis.

The streptococci, when present in the cultures, have not, as a rule certainly, entered with the ascitic fluid or the kidney fragment or from the air. There is no reason to doubt that they have been introduced with the fragment of nervous or other tissue with which cultivation was attempted. Whether they are also to be regarded as contaminations in the broad sense is the question at issue.

A summary of all the extraneous microorganisms encountered (Table III) reveals that a large proportion was streptococci. It is of interest to have found that the tubes prepared from the liver and

kidney of the monkeys were more frequently attended by growth of streptococci, and other extraneous organisms, than those of the spleen, and the tubes of the spleen more often showed extraneous organisms than those of the brain.

In three monkeys there was a rich growth of a pure culture of streptococci in all the tubes of the first generation, the control tubes remaining sterile. In all these instances the animal had been prostrate 24 hours or longer, and in two instances had been dead several hours when the autopsy was performed. This finding is in such definite contrast with the results of the cultures prepared from the corresponding tissues of monkeys severely paralyzed but not yet prostrate and moribund or dead that it would seem to throw considerable

TABLE III.

Summary of Contaminations with Streptococci and Other Organisms in Poliomyelitic Cultivation Experiments.

Tissue.	No. of original culture tubes made.	No. of tubes contaminated with streptococci in 1st generation.	No. of tubes contaminated with streptococci in all later generations.	No. of tubes contaminated with organisms other than streptococci in all generations.
Brain	137	25	11	10
Spinal cord	14	0	7	0
Kidney	31	7	5	0
Liver	31	5	2	3
Spleen	30	6	1	2
Adrenal	17	3	1	1
Mesenteric node	19	2	0	0
Thymus	10	2	0	0
Pancreas	4	0	0	0
Total	293	50*	27†	16

* Forty-six of the fifty tubes that were contaminated with streptococci in the first generation were obtained from four monkeys that had been prostrate and moribund for a considerable time before autopsy. The remaining four streptococcic contaminations in the first generation of tubes were from fourteen other monkeys, autopsied under more favorable conditions.

† An average of more than five subcultures from each original tube was made so that the total number of contaminations with streptococci in all subsequent generations (27) represents the extraneous contaminations in over 1,000 tissue culture tubes.

light on the nature of the streptococcal invasion, detected by Mathers (5), Rosenow, Towne, and Wheeler (6), and Nuzum and Herzog (7) in human beings and monkeys who have succumbed to poliomyelitic infection.

In order to obtain more light on this subject, we cultivated, by means of the technique employed for the globoid bodies, the tissues of monkeys which had succumbed in the laboratory to tuberculosis, dysentery, and other diseases, and isolated, in several instances, streptococci from the liver, kidney, spleen, and nervous tissues.

The streptococci yielded by the tissues of the moribund and dead poliomyelitic monkeys were transplanted into carbohydrate media and injected into rabbits. The fermentation reactions were those of streptococci in general and sharply distinguished them from the globoid bodies. The inoculations gave results so closely in accordance with those described by Bull (8) as not to call for restatement here.

Typical Cultivation Experiments.

Experiment A.—February 23. *Macacus cynomolgus.* Inoculated into left cerebral hemisphere with 0.1 cc. of an N Berkefeld filtrate of a centrifuged 5 per cent suspension of glycerolated brain and spinal cord carrying mixed virus. March 4. Tremor of head, slow movements, weakness of arms. March 5. Extensive paralysis; animal unable to rise. Etherized and bled to death from the heart. The autopsy showed the presence of typical lesions of poliomyelitis.

Cultures were made as follows: brain, 8 tubes; spinal cord, 4 tubes; intervertebral ganglion, 1 tube; kidney, 3 tubes; liver, spleen, thymus, 2 tubes each. Rabbit kidney fragments from a single animal were employed, but two specimens of ascitic fluid were used. The even-numbered tubes received ascitic fluid, Lot 7, the odd-numbered tubes Lot 10. The control tubes consisted of ascitic fluid plus kidney, ascitic fluid alone, and a kidney tissue-ascitic fluid inoculated with stock culture of globoid bodies No. 973. All the tubes were placed in the hydrogen-nitrogen jar and incubated, and in addition three tubes of ascitic fluid plus kidney and brain were incubated in the open air and examined every other day. The latter series remained sterile and was discarded on the 8th day.

March 15. Hydrogen jar opened. Sodium pyrogallate solution was colorless; hence the jar had been oxygen-free. Fresh kidney tissue-ascitic tubes were reinoculated from each of the original tubes except the thymus tube. The fragment of thymus had floated to the top, and the tube was discarded. Rabbit kidney No. 9 was used for the new tubes; and for the even-numbered tubes ascitic fluid No. 7 and in the odd-numbered tubes ascitic fluid No. 6 were used. The usual controls were added and all placed as before in the anaerobic jar.

March 17. The microscopic examination of the first generation tubes was negative throughout.

March 26. The second anaerobic jar had remained oxygen-free. In removing the tubes, spleen tube No. 20 and brain tubes Nos. 7 and 8 were broken. Microscopic examination of the remaining intact tubes gave the following result:

Brain Tube No. 1.—Organisms suggestive of globoid bodies.

Spinal Cord Tubes Nos. 10 and 12, Ganglion Tube No. 13, and Spleen Tube No. 19.—Indefinite bodies, somewhat suggestive of the globoid bodies.

Cord Tube No. 11 and Liver Tube No. 17.—Grossly contaminated.

March 26. Ascitic fluid-kidney tissue tubes were inoculated from the second generation, using ascitic fluid No. 13 for the odd-numbered and No. 6 for the even-numbered tubes. Anaerobic cultivation.

April 4. The jar had remained free of oxygen. Microscopic examination of the tubes gave the following result:

Brain Tube No. 1.—No growth.

Brain Tube No. 5.—Same as No. 1.

Other tubes of the third generation suggestive of globoid bodies were: brain No. 6, cord No. 12, and kidney No. 16.

April 4. Tubes of semisolid medium were inoculated and placed in the anaerobic jar.

April 10. Jar opened. All the cultures were negative except brain No. 6, cord No. 12, intervertebral ganglion No. 13, and spleen No. 19, which were merely suggestive, and cord No. 10, which contained a growth of the globoid bodies. Transplantation from the tubes which were suggestive and the one positive to fresh tubes of semisolid medium gave no result. Thus far the cultivation experiments with this specimen of virus, which had been carried through 7 or 8 weeks, resulted in three cultures of the globoid bodies which could not be developed in the fourth generation.

The ascitic fluid-kidney tissue culture of March 26 was preserved in the refrigerator for 20 days. Transplantation from it was again made into fluid and semisolid media. Brain tube No. 1 gave a positive growth which again failed to grow in the next transplantation. But by returning again to the fluid culture of March 26, a strain of brain No. 1 culture was secured which continued to grow in semisolid medium. Similarly the ascitic fluid-tissue tube of cord No. 12, made on April 14 from the mother tube of March 26, was positive and yielded a culture capable of growing in subcultures.

Thus from a total of more than 100 tubes carried over a period of more than 2 months and through five or six generations, two strains of the globoid bodies which bore subculturing were finally obtained. In each instance the organism was detected in the second generation; but patience and persistence were needed to obtain strains which would continue to grow in artificial media. All the cultivation ex-

periments were not so long, tedious, and difficult as this one; but it has been given in detail to illustrate the intricacy of the problem of the cultivation of the globoid bodies.

Experiment B.—April 15. *Macacus rhesus.* A cotton plug, containing 1 gm. of fresh mixed virus, was placed in the left nares, where it was allowed to remain for 16 hours. April 22. Right arm was paralyzed. April 25. Paralysis has slowly progressed; animal was almost prostrate but was bright. April 27. Extensive paralysis; complete prostration. Etherized and bled from the heart. Autopsy showed the presence of typical lesions of poliomyelitis in the cord and brain.

Cultures were made as follows: Brain, 8 tubes; kidney, 2 tubes; spleen, 3 tubes; adrenal, 2 tubes; liver, 3 tubes; mesenteric nodes, 2 tubes; and pancreas, 2 tubes; a total of 22 tubes. Three lots of ascitic fluid were used and kidney tissue from two rabbits, the same ascitic fluid being placed in every third tube and the same rabbit kidney tissue in every alternate tube. Controls were made as usual. The hydrogen-nitrogen jar was used and was satisfactory throughout the experiment.

May 8. The anaerobic jar was opened and all tubes were found to be in good condition except one liver tube, which was broken. A complete set of tissue tubes was made up, one rabbit kidney and two fresh ascitic fluids being used. These were placed in the hydrogen-nitrogen jar as before. Microscopic examination of the tubes of the first generation showed no growth in any tube.

May 20. The anaerobic jar of May 8 was opened. Examination of the tubes revealed a typical microscopic picture of the globoid bodies in brain tubes Nos. 1 and 5. One spleen tube and one liver tube were contaminated with a large Gram-positive bacillus and were discarded. Semisolid tissue culture tubes were made from each of the remaining tissue culture tubes of the second generation and placed in a hydrogen-nitrogen jar.

May 29. The anaerobic jar of May 20 was opened. A few tiny colonies were to be seen in the bottom of the following tubes: brain No. 4, brain No. 5, brain No. 6, spleen No. 12, spleen No. 13, and adrenal No. 11. Microscopic examination confirmed the presence of the globoid bodies. There was no growth in the other tubes, and they were discarded.

Fresh semisolid tissue tubes were at once made of all the suggestive semisolid cultures of the third generation and also from the ascitic fluid tubes of the second generation, which had been kept in the ice box since May 20. These tubes were all placed in the anaerobic jar as usual.

The fourth generation tubes resulted in the definite establishment of four brain strains, three from the semisolid media of May 29 and one from the ascitic fluid media of May 20. The two spleen strains were also definitely established from the spleen semisolid tubes of May 29. Adrenal tube No. 11 yielded a typical growth in the third generation, but it refused to grow in all subsequent generations and therefore cannot be included in the series.

In this experiment, therefore, a total of six positive cultures was obtained, four from the brain and two from the spleen, out of a total of twenty-two original tubes. There were two contaminations in the series, both occurring in the second generation.

Carbohydrate Reactions of the Globoid Bodies.

In their original publication Flexner and Noguchi (1) state that the globoid bodies have no ability to split the polysaccharides, alcohols, or even the simple hexoses. Since it is obvious that if an organ-

TABLE IV.

Reaction of the Globoid Bodies upon Simple Sugars.

Strains.	Strain No.	Dextrose.		Lactose.	
		Titration to phenolphthalein in the cold.	pH	Titration to phenolphthalein in the cold.	pH
New strains.	5	0.60	7.3	0.72	7.3
	6 (Brain No. 1).	0.56	7.3	0.60	7.2
	6 (Cord " 12).	0.62	7.3	0.62	7.3
	7	0.52	7.25	0.50	7.3
	8	0.50	7.3	0.64	7.2
	12	0.64	7.2	0.68	7.2
	4 (Brain No. 3).	0.46	7.3	0.64	7.2
	4 (Spleen " 13).	0.56	7.3	0.58	7.3
Stock strains.	1281	0.56	7.2	0.48	7.3
	1328	0.52	7.2	0.60	7.2
	973	0.56	7.3	0.70	7.3
Controls. Streptococci.*	1623–2	6.6	5.3	5.6	5.6
	1556	4.56	5.8	3.1	6.0
Controls.					
Kidney + ascitic broth.		0.60	7.1	0.62	7.2
Ascitic broth alone.		0.54	7.25	0.54	7.3

Titration results are expressed in the number of cc. of 0.1 N sodium hydroxide which would be required to neutralize 100 cc. of the medium.

The hydrogen ion concentrations were done by the Henderson-Palmer colorimetric method (9).

* Both strains of streptococci were obtained from the first generation of brain tissue tubes from poliomyelitic monkeys.

ism is unable to affect simple sugars, it will also be unable to affect higher ones, the first necessary experiment is to determine the reaction of the organism upon simple sugars.

In our experiments dextrose and lactose only were used. The medium employed is described in the paragraphs upon technique. There are so many buffer salts in an ascitic bouillon medium that the determination of acid production by titration with 0.05 N sodium hydroxide is not satisfactory. Instead, one should use one of the various methods for the determination of the hydrogen ion concentration.

Eleven strains of the globoid bodies were inoculated into the sugar media. Eight of them had been isolated within the past 3 months, and three were stock cultures that had been cultivated in the laboratory for one or more years. Controls were added to the series of two strains of streptococci which, as contaminations, had been isolated from poliomyelitic monkeys during the previous 3 months. Controls also were added of tubes containing ascitic broth plus rabbit kidney tissue and ascitic broth alone. The tubes were incubated in an anaerobic jar for 7 days, at the end of which time there was a good growth in each tube. The results of the experiment are summarized in Table IV. Thus none of the globoid bodies were able to attack the simple sugars, while the two strains of streptococci attacked them vigorously.

Inoculation of Monkeys with Cultures of the Globoid Bodies.

That the inoculation of pure cultures of the globoid bodies, even in a remote generation, will sometimes produce infection in monkeys, attended by the symptoms and specific lesions of experimental poliomyelitis, has been shown by the reports of Flexner and Noguchi (1), and Flexner, Noguchi, and Amoss (10). On the other hand, their experiments indicate that it is exceptional for the cultivated globoid bodies to exhibit definite pathogenic properties. However, the observation was made that in some animals in which a single inoculation failed to cause any symptoms, a subsequent one was followed by typical paralysis attended by the specific lesions in the central nervous organs of the infected monkeys.

The question naturally arose whether any of the cultures of the globoid bodies isolated by me possessed pathogenic properties. Eight different strains were inoculated into *Macacus rhesus* monkeys. In some instances a single injection, in others several injections were given. Three of the inoculated animals developed some degree of paralysis following intracranial or intraspinal inoculation. In one instance the culture was in the fourth, in the remaining two in the fifth generation. The other five animals did not develop suspicious symptoms. A brief recapitulation of the protocols of the three monkeys showing paralysis is given, followed by a discussion of the significance of the experiments.

Experiment C.—Macacus rhesus. May 19. Left intracerebral inoculation of 2 cc. of an ascitic fluid culture M.A. in the fifth generation of the globoid bodies derived from the brain of a monkey. May 25. Animal had a convulsion, following which it was ataxic and dazed. Lumbar puncture yielded a turbid fluid under pressure containing 1,400 cells, chiefly lymphocytes, and ++ globulin with 0.1 cc. May 26. Left arm protected; slight left facial palsy; ataxia. May 28. Facial paralysis marked. Following lumbar puncture brief convulsion. Cerebrospinal fluid clearing; excess of lymphocytes. May 31. Condition improved; probably will recover; etherized for cultures and histology. The autopsy revealed a cyst at the point of inoculation, containing yellow fluid. A filtrate of the local site and an emulsion of the spinal cord and medulla were inoculated into two *rhesus* monkeys, respectively; neither developed symptoms.

Cultures were prepared from the brain, kidney, liver, spleen, mesenteric node, and adrenals. All tubes were carried through three generations without contamination. The globoid bodies were isolated from one tube only, brain tube No. 2.

Sections were prepared from the medulla, cervical and lumbar regions of the spinal cord, and intervertebral ganglia. In none were any lesions characteristic of poliomyelitis found.

The globoid bodies employed for inoculation were derived from the M.A. virus, which, in its present condition, is of low virulence for monkeys. The inoculation gave rise to a local cyst, which rarely results from the intracerebral inoculations, attended by a distinct left facial palsy and weakness (?) of the left arm. It is doubtful whether the symptoms denoted experimental poliomyelitis. Probably the facial palsy was related to the cyst formation at the inoculation site; as definite paralysis did not appear in the arm, the appearance of

weakness may have been deceptive. The complete absence of histological lesions is inconsistent with the production of typical experimental poliomyelitis; although since the only developed paralysis was facial, it is always possible that exhaustive microscopic study might have revealed poliomyelitic lesions in the nucleus of the facial nerve. However, the failure of the filtrate and tissue emulsion to transmit the disease to other monkeys also speaks against the condition having been experimental poliomyelitis.

On the other hand, the injection of the globoid bodies gave rise to a marked cellular reaction in the cerebrospinal fluid, in which the predominating cells were lymphocytic. Ordinary bacteria which set up a meningitis usually produce polymorphonuclear cells. But the chief point of importance is the great difficulty and slight success attending the recovery, by cultivation, of the globoid bodies, even from the inoculated nervous tissues. Once these cultivated microorganisms readapt themselves to the condition of growth within the living body, they resist artificial cultivation as do the original tissue parasites—a point noted in Flexner and Noguchi's first communication.

Experiment D.—Macacus rhesus. June 9. Inoculated into the left cerebral hemisphere with 2 cc. of a lightly centrifuged unwashed sediment of a 5 day old mass culture of the globoid bodies isolated from the spleen of a monkey. The strain of the globoid bodies had passed through five generations and been under cultivation for 55 days. This animal developed convulsions, ataxia, and weakness of arms and legs. A lumbar puncture performed on June 14 yielded fluid not under pressure, containing ++ globulin 0.1 cc. and 110 mononuclear cells per c. mm. June 24. The symptoms had all diminished. The animal was given 2 cc. of a culture of the globoid bodies from the spleen intraspinally and 4 cc. intraperitoneally. The only result of this injection was to bring about a temporary increase of the ataxia. Recovery finally became complete.

While a definite reaction was obtained in this instance, there is doubt whether the symptoms really indicated the production of experimental poliomyelitis.

Experiment E.—Macacus rhesus. June 2. An intraspinal injection was made of 2 cc. of a mass M.A. culture in the fourth generation. June 7. The animal was somewhat ataxic and excitable, and the right arm was protected. June 18. The animal was distinctly weak and tended to fall to the left side. Etherized. The autopsy revealed a generalized tuberculosis of the viscera. A few tubercles were detected in the meninges.

The histological examination of the spinal cord, medulla, brain, and intervertebral ganglia revealed no poliomyelitic lesions. A miliary tubercle was present in the intima of a small vein in one of the ganglia.

In other words, aside from the symptoms suggestive of experimental poliomyelitis, no pathologic basis for the diagnosis could be obtained. The experiment should be regarded as negative.

If we review the results of the inoculation of monkeys with cultures of the globoid bodies, we must conclude that, while certain symptoms suggestive of poliomyelitis were sometimes produced, in no instance was the experimental disease, as determined by the presence of typical lesions in the nervous organs, actually set up. Our experiments confirm, therefore, the conclusion arrived at by Flexner and Noguchi, that it is the very exceptional cultures only which retain pathogenic power sufficient to cause infection in monkeys.

DISCUSSION.

The presentation of this work on the cultivation of the globoid bodies may be considered from several points of view. Perhaps the first point that should be discussed is that relating to the ultimate results achieved. The experiments show clearly, I think, that if the experimenter has suitable poliomyelitic tissues to work on and suitable samples of ascitic fluid, and if the inoculated tubes are kept under strict anaerobic conditions, and transferred at proper intervals, successful results, in some degree, will almost surely follow. In fact, the decision arrived at was to the effect that it may be possible to cultivate the globoid bodies from practically all cases from which suitable material is available. If, for example, the ascitic fluid available during the period of May 15 to June 1 had been suitable, the globoid bodies present in the fluid medium could probably have been grown in the semisolid medium. Because of this failure, I can report the cultures merely as suggestive instead of as positive. Similarly, in other experiments (Nos. 3 and 4, Table II), it is highly probable that growth in the semisolid medium would have taken place had the hydrogen-nitrogen jar been employed instead of the vacuum jar.

However, the introduction of the hydrogen-nitrogen jar has not removed the chief drawbacks of the method; namely, the personal factor of painstaking care and perseverance. At best the methods

are long, tedious, often discouraging, requiring now and again months of time, abundance of suitable material, and an exact technique in order to succeed even in one experiment. I do not consider that I have modified fundamentally the original Noguchi technique. I have devised an alternative method which seems to possess certain advantages. And yet I never succeeded in identifying the globoid bodies in the first generation, although the original investigators did so.

The chief difficulty encountered is the establishment of the strain. It would appear either that the more pathogenic of the organisms do not develop in the artificial cultures, or that when they develop they do so at the expense, as a rule, of their power to produce infection. In this respect they may be said to resemble *Treponema pallidum*, with which they share so many cultural requirements and immunological reactions, as has previously been pointed out by others (1, 11).

After having once become established and accustomed to the artificial media, the globoid bodies grow more readily, may be more easily transferred, and will survive at refrigerator temperature for months. There are certain limitations, however, beyond which they will not go. Body fluids, preferably ascitic fluid, are required for their development, and strict anaerobiosis is also essential. Furthermore, the reaction of the culture media must be at approximately blood neutrality, and indicators, such as litmus, neutral red, Andrade's, etc., inhibit their growth.

The results obtained from the cultures of organs other than nervous tissues were unsatisfactory. The great obstacles to success with them appear to be contaminating organisms, particularly the streptococcus. This common microorganism appears to be more frequently present in the general viscera than in the central nervous organs; and the cultures prepared from the kidney and liver are the ones most often developing streptococci. Apparently also the globoid bodies are present less constantly or in smaller numbers in the general viscera. Finally, despite the exsanguination of the animal, the visceral tissues always contain a certain amount of blood, which probably interferes with the development of the globoid bodies in the initial culture tubes.

Despite these adverse factors, the successful cultivation of the organ-

ism from the spleen shows that it is contained outside the nervous and in the lymphatic visceral organs. Moreover, in four other instances, the globoid bodies were cultivated from the visceral tissues in the fluid medium, although they could not be developed in the semisolid medium. These strains included one from the adrenal of the animal yielding the spleen culture, another spleen culture, one from the kidney, and one from the mesenteric lymph node of two other monkeys. The experiments are the first reported in which the globoid bodies have been cultivated from tissues other than those of the central nervous system.

The inoculation of monkeys with the cultures should be regarded as having failed to produce the experimental poliomyelitis. The circumstances surrounding the failures are in themselves instructive. Had the deductions been based merely on the clinical symptoms, they would have pointed to the induction of the infection. The ultimate criteria of experimental poliomyelitis are (1) the typical histological lesions and (2) recommunicability of the disease by inoculation of the nervous tissues of the suspected case. Since neither of these could be satisfied in the experiments, I regarded them as negative.

CONCLUSIONS.

The globoid bodies, identical in morphological and cultural characteristics with the organisms described by Flexner and Noguchi, have been obtained in twenty-two cultures from the tissues of seven monkeys suffering from experimental poliomyelitis.

Twenty of the strains were cultivated from the central nervous organs, all being obtained from the cerebrum except one, which was cultivated from the cervical portion of the spinal cord.

Two strains were cultivated from the spleen.

None of the cultivated strains inoculated produced typical poliomyelitis in monkeys.

The recovery of a strain of the globoid bodies from the inoculated monkey is as difficult as is the original cultivation of the organisms from animals inoculated with the ordinary virus of poliomyelitis.

Nothing in this study has served to implicate the streptococcus in the pathology of the poliomyelitic process; the streptococcus is, how-

ever, encountered as a common contaminant or secondary invader, especially in animals which have been etherized while moribund, or which had died some hours previous to the autopsy. When the infected and paralyzed animals are killed while still strong, secondary invading bacteria, including the streptococcus, tend to be absent from the tissues.

A modified, perhaps improved, but alternative method has been devised for the cultivation of the globoid bodies and other microorganisms demanding a high degree of anaerobiosis.

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THE RELATION OF CIRCULATING ANTIBODIES TO SERUM DISEASE.

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Though all phases of anaphylaxis have been studied in animals, comparatively few systematic investigations have been made upon the condition as it occurs in man. Moreover, different species vary in their susceptibility to artificial sensitization of foreign proteins as well as in their reactions to a second injection of protein, and hence it is highly essential when opportunities arise to study in detail the problems of anaphylaxis in man.

In this connection the essential difference between man and the experimental animals is that the primary sensitizing dose of serum in animals is not followed by any obvious symptoms, whereas in man it frequently results in serum disease. The object of this investigation was to determine what relation, if any, existed between the formation of antibodies to horse serum and the course of serum disease.

During the last few years we have had an opportunity to study this problem in twenty-five individuals who, for therapeutic purposes, have received various quantities of antitoxic or antibacterial horse serum. Of the twenty-five cases, twenty-one suffered from pneumonia and received repeatedly large doses of antipneumococcus horse serum intravenously.[1] The smallest amount administered to one patient was 5 cc., the largest 630 cc., and the average amounts varied from 180 to 360 cc. Of the remaining four cases, one of meningitis received 40 cc. of antimeningococcus serum intraspinally, one

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[1] This serum was kindly furnished by Dr. Rufus Cole of the Hospital of The Rockefeller Institute for Medical Research and was an antiserum for Pneumococcus Type I.

case of tetanus received 22.5 cc. of antitetanus serum intraspinally, and two cases of diphtheria each received 7.5 cc. of the antitoxin subcutaneously.

To demonstrate the antisubstances to foreign protein in animals and occasionally in man, three methods have heretofore been employed: first, the test for precipitins; second, the demonstration of anaphylactin by the passive transfer of anaphylaxis by the blood serum of the test animals to a normal guinea pig; and third, the presence of a specific skin reaction. Since the classic studies of von Pirquet and Schick upon serum disease (1), a number of investigations have been made upon the relation that one or another of these reactions bears to the development of serum disease, but without definite results as regards their significance.

Hamburger and Moro (2), who first studied the precipitin reaction in children following injections of diphtheria antitoxin, found that the reaction appeared during serum disease, and as the precipitin increased in concentration in the blood the precipitinogen or horse serum decreased. They concluded that the interaction of precipitin and horse serum in the circulation produced a poisonous substance that was responsible for serum disease. Von Pirquet and Schick could find no relation between the presence of precipitins in the circulation and serum disease. Lemaire (3) confirmed the results of Hamburger and Moro, and stated, further, that precipitins were not demonstrable unless serum disease followed the injections of antitoxin. Wells (4), in a study of the precipitin content of the blood of children to whom diphtheria antitoxin had been administered, found precipitin in the circulation occasionally before the onset of serum disease. During serum disease precipitin was present in low concentration but rose rapidly in concentration towards the end of the illness to fall gradually thereafter and eventually disappear. Weil (5) has made similar observations.

Regarding the development of anaphylactin during serum disease, little is known. The isolated observations of Rosenau and Anderson (6), Anderson and Frost (7), Achard and Flandin (8), and Weil (9) show that anaphylactin to horse serum may be transferred passively from man to guinea pig. Novotny and Schick (10), however, succeeded in demonstrating anaphylactin in the blood of only two of twelve children that had received injections of horse serum. Grysez and Bernard (11), on the other hand, were able to sensitize guinea pigs passively to horse serum with the blood drawn from eleven children 5 to 243 days after the injection of horse serum.

Doerr and Russ (12), Doerr and Moldovan (13), and recently Weil (14) have brought forth evidence to show that the precipitating substance and anaphylactin are one and the same, but since it has also been shown both by Doerr and Moldovan and by Weil that serum may transmit passive anaphylaxis and yet

show no precipitating properties, it is necessary in any investigation upon the development of antibodies in serum to take into account the presence of both these reactions. It is possible, moreover, that the anaphylactic antibody which these observers find in the precipitate formed when antigen and antiserum are brought together may not be identical with the precipitate but absorbed by it during the process of precipitation. That protective antibodies may be carried down from antiserum by precipitates is well known,[2] and it is possible that the same may be true of anaphylactic antibody.

The occurrence of the specific skin reaction during and after serum disease has been observed by Hamburger and Pollak (15), Michiels (16), and Cowie (17). Michiels found that the reaction could rarely be obtained during serum sickness though it appeared shortly after the cessation of the illness. Cowie, by making repeated intradermal inoculations of 0.5 cc. of horse serum in children who had received immunizing doses of diphtheria antitoxin, obtained a positive skin reaction in one case on the 4th day, in seven cases on the 5th day, and in nine cases between the 6th and 10th days after the injection of antitoxin.

In the present investigation the three methods of study already discussed have been employed, though in only four cases have they all been applied to the same individual. In order to determine the time of appearance and the relation of these reactions to serum disease, observations were made at short intervals before, during, and after serum disease in all cases. The curves of precipitin formation were studied in fourteen cases, the development of anaphylactin in fifteen, and the appearance of the skin reaction in fifteen cases.

The precipitin reaction was carried out as follows: To 0.5 cc. of horse serum undiluted and in dilutions up to 1: 100,000 in isotonic salt solution, the patient's serum was added in quantities of 0.5 cc. As controls horse serum was titrated against anti-horse rabbit serum and normal human serum in similar amounts, while sheep serum and beef serum were titrated against the patient's serum. The tubes were kept at 37°C. for 1 hour and then allowed to stand over night in the ice box, when the final reading was made.

In attempts to transmit anaphylactin from one species to another cognizance must be taken of the fact demonstrated by Weil (18) and by Lewis (19) that when two different antigens or foreign proteins are simultaneously injected into an animal the protein in excess

[2] For a discussion of this subject see Gay, F. P., and Chickering, H. T., *J. Exp. Med.*, 1915, xxi, 389.

may prevent either active or passive sensitization to the other protein. It has also been repeatedly observed in attempts to transmit anaphylaxis passively that small amounts of serum will give positive results when the experiment fails with larger quantities. These facts soon became obvious in the present investigation and, therefore, in each experiment a series of guinea pigs was injected with varying quantities of the patients' serum.

The blood drawn from the patient was centrifugalized immediately, the serum separated, heated at 56°C. for 30 minutes, and injected intraperitoneally into four to eight guinea pigs in doses varying from 0.1 to 3 cc. In the majority of instances four doses were employed: 0.1 cc., 0.5 cc., 1 cc., and 3 cc. After an interval of 18 to 30 hours an intravenous injection of 0.5 cc. of horse serum was given to each guinea pig. Definite symptoms of anaphylactic shock were required before the result was termed positive.

To test the skin reaction 0.02 to 0.05 cc. of 1:10 and 1:100 dilutions of horse serum was injected intradermally by means of a specially graduated glass syringe. In the normal individual these minute quantities of serum may occasionally give rise to an immediate reaction. This consists in the appearance within 30 seconds to 1 minute of a red areola 1 to 3 cm. in diameter which rapidly fades within the next 5 minutes and is succeeded by a white indurated wheal 5 to 7 mm. in diameter. This may persist for 15 to 20 minutes. Since the same reaction may be obtained with various fluids, such as isotonic salt solution and water, it was considered by us as a traumatic reaction. In the individual sensitized to horse serum the traumatic reaction may or may not occur. Under any circumstances the true reaction makes its first appearance in 10 to 15 minutes. At this time the original wheal rapidly grows, becomes firm, yellowish in color, often itches, and at the same time a red areola spreads from the margin of the wheal until after 20 to 30 minutes the wheal reaches 15 to 20 mm. in diameter and the areola 30 to 50 mm. in diameter. Within half an hour the reaction begins to fade and after 1 to $1\frac{1}{2}$ hours subsides.

To follow the development of this reaction intradermal injections of diluted horse serum were always made before the therapeutic injections of horse serum and at frequent intervals afterwards until

strongly positive reactions were obtained. As controls, sheep and beef serum in the same quantities and isotonic salt solution were employed.

Of the twenty-five cases which were studied twenty-one developed serum disease that was manifest by such characteristic symptoms as enlargment of lymph nodes, skin eruptions, edema, fever, arthralgia, and enlargement of the spleen. The disease varied from a mild form (Case 11) to a severe relapsing form extending over a period of 3 weeks (Cases 1 and 2). In three cases there were no evidences of serum disease (Cases 12, 14, and 23), and in one patient (Case 13) who received three intraspinal injections of tetanus antitoxin, there were no other signs than slight enlargement of the lymph nodes.

Precipitins.

Of the fourteen cases in which the serum was tested for precipitins, twelve developed serum disease. In all but one of these, precipitins could be demonstrated on one and usually several occasions. Once having made their appearance in the serum they persisted, as a rule, for days and often weeks. In one patient who received 520 cc. of horse serum intravenously in divided doses precipitins could be demonstrated on the 34th day after their first appearance in such concentration that 0.5 cc. of the patient's serum formed a precipitate with 0.5 cc. of a 1: 100,000 dilution of horse serum. The serum from another patient who received 630 cc. of horse serum in divided doses contained precipitins for 58 days. In most instances the patient was discharged from the hospital before the precipitin reaction had disappeared. In two cases precipitins were demonstrated on one occasion only.

In the two patients who escaped serum disease, precipitins could not be demonstrated in the blood at any time. One patient received 300 cc. of horse serum in divided doses, the other 240 cc.

Anaphylactin.

Of the fifteen patients whose blood was tested for anaphylactin, twelve developed serum disease. With the serum of all but one of the latter patients it was possible on one or more occasions to

transmit anaphylaxis passively to guinea pigs. As a rule, the presence of anaphylactin in the circulation was of short duration and positive reactions could be obtained only once or twice. A typical example is shown in the protocol in Table I.

In one instance, however, positive reactions were obtained on five

TABLE I.

Passive Transfer of Anaphylaxis for Horse Serum from Patient to Guinea Pigs.

W. B.; lobar pneumonia. Feb. 6, 1916. 80 cc. of antipneumococcus horse serum intravenously.

Feb. 7. 80 cc. of antipneumococcus horse serum intravenously.

Feb. 17. Onset of serum disease continuing until Feb. 26.

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.	
	gm.	*1916*			*cc.*	*cc.*			
1	357	Feb. 11	5		0.5	0.5	26 hrs.	+ Recovery.	+
2	348	" 11	5		0.5	0.5	26 "	No symptoms.	
3	300	" 11	5		3.0	0.5	26 "	+ Recovery.	
4	205	" 11	5		3.0	0.5	26 "	No symptoms.	
5	Positive control. Jan. 12. 0.1 cc. of horse serum intravenously.					0.5	31 days.	+++ Dead in 3 min.	
6	195	Feb. 17	11	½	0.1	0.5	27½ hrs.	No symptoms.	−
7	204	" 17	11	½	0.5	0.5	27½ "	" "	
8	200	" 17	11	½	1.0	0.5	27½ "	" "	
9	210	" 17	11	½	3.0	0.5	27½ "	" "	
10	420	Positive control. Nov. 18, 1915. 0.25 cc. of horse serum intravenously.				0.5	92 days.	+++ Dead in 3 min.	
11	235	Feb. 21	15	4	0.1	0.5	28 hrs.	No symptoms.	++
12	210	" 21	15	4	0.5	0.5	28 "	" "	
13	183	" 21	15	4	1.0	0.5	28 "	++ Very slow recovery.	
14	217	" 21	15	4	3.0	0.5	28 "	+++ Dead in 12 hrs.	
15	299	Feb. 27	21	10	0.1	0.5	25 hrs.	No symptoms.	−
16	357	" 27	21	10	0.5	0.5	25 "	" "	
17	348	" 27	21	10	1.0	0.5	25 "	" "	
18	310	" 27	21	10	3.0	0.5	25 "	" "	
19	Positive control.					0.5		++ Recovery.	

TABLE II.

Passive Transfer of Anaphylaxis for Horse Serum from Patient to Guinea Pigs.

W. N.; age 12 years; meningitis. Dec. 11, 1915. Intraspinal injection of 20 cc. of antimeningococcus serum.

Dec. 12. Intraspinal injection of 20 cc. of antimeningococcus serum.

Dec. 14. Onset of serum disease continuing until Dec. 22.

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.
	gm.	*1915*			*cc.*	*cc.*	*hrs.*	
20	218	Dec. 16	5	2	0.1	0.5	26	No symptoms.
21	234	" 16	5	2	0.1	0.5	26	" "
22	235	" 16	5	2	0.25	0.5	26	" "
23	219	" 16	5	2	0.25	0.5	26	" "
24	267	Dec. 18	7	4	0.15	0.5	39	No symptoms.
25	302	" 18	7	4	0.20	0.5	39	" "
26	242	Dec. 21	10	7	0.1	0.5	22	+++ Recovery.
27	197	" 21	10	7	0,1	0.5	22	+++ "
28	182	" 21	10	7	1.0	0.5	22	+++ "
29	216	" 21	10	7	1.0	0.5	22	+++ "
		1916						
30	205	Jan. 3	23	20	0.1	0.5	22	+(?) Recovery.
31	245	" 3	23	20	0.5	0.5	22	++++ Dead in 4 min.
32	170	" 3	23	20	1.0	0.5	22	++++ " " 2 "
33	250	" 3	23	20	3.0	0.5	22	No symptoms.
34	250	Positive control. Nov. 18, 1915. 0.3 cc. of horse serum intravenously.				0.5		++++ Dead in 2 min.
35	257	Jan. 18	38	35	0.1	0.5	20	No symptoms.
36	286	" 18	38	35	0.5	0.5	20	" "
37	227	" 18	38	35	1.0	0.5	20	+(?) Recovery.
38	297	" 18	38	35	1.0	0.5	20	+(?) "
39	305	" 18	38	35	2.5	0.5	20	+++ "
40	236	" 18	38	35	3.0	0.5	20	++++ Dead in 8 min.
41	335	Positive control. Dec. 7, 1915. 0.5 cc. of horse serum intravenously.				0.5		++++ " " 2 "
42	247	Feb. 2	53	50	0.1	0.5	20	No symptoms.
43	234	" 2	53	50	0.1	0.5	20	" "
44	265	" 2	53	50	1.0	0.5	20	++ Recovery.
45	218	" 2	53	50	3.0	0.5	20	+++ "

TABLE II—*Concluded.*

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.
	gm.	1916			*cc.*	*cc.*	*hrs.*	
46	199	Feb. 21	72	69	0.1	0.5	28	No symptoms.
47	252	" 21	72	69	0.5	0.5	28	+ Recovery.
48	202	" 21	72	69	1.0	0.5	28	++ "
49	188	" 21	72	69	3.0	0.5	28	+++ "
50	302	Mar. 9	89	86	0.5	0.5	22	No symptoms.
51	270	" 9	89	86	1.0	0.5	22	" "
52	315	" 9	89	86	3.0	0.5	22	" "
53	Positive control. Jan. 2. 0.5 cc. of horse serum intravenously.					0.5		++++ Dead in 4 min.

occasions from the 10th to the 72nd day after the administration of 40 cc. of horse serum intraspinally. Table II gives the protocols of these experiments.

In three cases the injections of serum were not followed by serum disease. Anaphylactin could never be demonstrated in the serum drawn repeatedly from these patients.

Skin Reactions.

In fifteen cases repeated skin reactions were made and in all of these a positive response was sooner or later observed. The skin reactions, unlike the temporary presence of precipitins and the fleeting reaction for anaphylactin, having once appeared, persisted and increased in intensity as long as the patients could be observed. The reaction was also observed in three patients who did not develop serum disease and whose blood serum showed no precipitin or anaphylactic antibody for horse serum. In these three cases the sensitiveness of the skin was much delayed in its onset. In the twelve cases that suffered from serum disease, the hypersensitiveness of the skin became evident on the 7th to the 15th day after the first injection of serum, whereas in these three patients who did not develop serum disease the first evidence of skin hypersensitiveness was observed in one on the 17th day, in one on the 33rd day, and in one on the 50th day, after the first injection of serum.

From these results it is evident that in the cases which we have studied precipitins and anaphylactic antibodies were much more likely to be found in the blood serum of patients who suffered from serum disease following the injections of horse serum than in patients who escaped serum disease. In the latter instances precipitins and anaphylactic antibodies were not observed. The skin reaction, however, made its appearance irrespective of the amount of serum administered or the method of administration and appeared whether or not serum disease developed.

The Relation of Antibody Formation to Serum Disease.

Further study was made in an attempt to determine whether there existed any direct relation between the formation of circulating antibodies and serum disease, and, if so, what this relation might be.

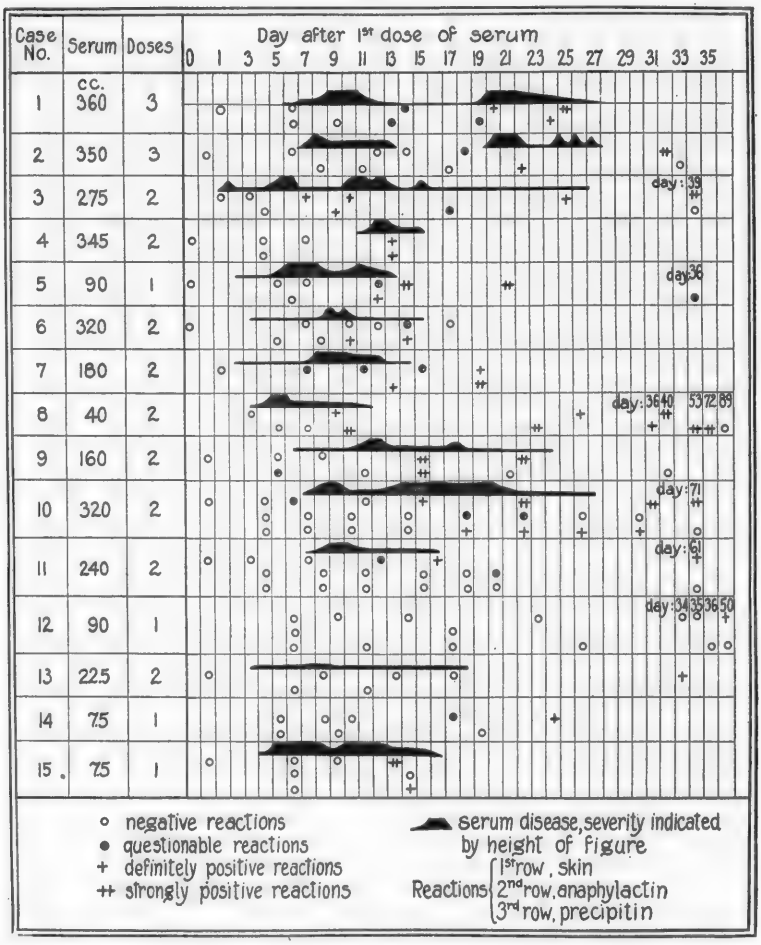
From the time that Hamburger and Moro demonstrated the presence of precipitin for horse serum in the blood of patients with serum disease, the question has constantly been raised as to whether these circulating antibodies play a part in the production of this illness. Von Pirquet and Schick were of the opinion that the illness depended upon the union of antibody and antigen either in the circulation or body cells and that the onset depended upon the proper balancing of these substances so that they might unite effectively to form a poison.

The problem may be viewed from three standpoints: (1) The formation of circulating antibodies may be totally independent of the serum disease. (2) The union of circulating antibodies and circulating antigen may produce a poison which gives rise to serum disease. (3) Antibodies may appear in the circulation as the result of a violent tissue reaction which manifests itself as serum disease, under which circumstances the antibodies might be protective rather than injurious.

The first proposition seems highly improbable, for otherwise one would expect antibodies to appear in the serum irrespective of serum disease, which was not true in this series of observations.

If the union of circulating antibody and circulating antigen were the sole cause of serum disease, one should be able to obtain evidence of the presence of these substances in the circulation immedi-

ately before or at the onset of serum disease. Such a demonstration has not been possible in these experiments. In only one instance (Table I) was a questionably positive result for anaphylactin obtained



TEXT-FIG. 1. The relation between the appearance of anaphylactin and precipitin in the circulation and of skin hypersensitiveness to serum disease in fifteen patients.

on the day previous to the onset of serum disease, and in no instance were precipitins present in the circulation before or at the time of onset of serum disease. Text-fig. 1 indicates the relation between the course of serum disease and the appearance of anaphylactin in the circulation.

It will be seen that blood serum drawn from the patients during the early stages of serum disease did not transmit anaphylaxis passively to guinea pigs. As a rule, anaphylactin was not demonstrable until the disease was well advanced or indeed about to terminate. This is particularly noticeable in Cases 1, 2, and 10, in whom relapses or recrudescences occurred. The blood from Case 1 did not give strongly positive results until the close of the relapse, which was 18 days after the onset of the disease. In Case 2 anaphylactin appeared 5 days before the close of the relapse and in Case 10 towards the subsidence of the recrudescence.

Table III is a composite table which shows even more strikingly

TABLE III.

The Relation between the Onset and Subsidence of Serum Disease and the Appearance of Anaphylactin in the Blood and of Hypersensitiveness of the Skin to Horse Serum, in Twelve Cases.

Days after 1st injection of horse serum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	38	50
Onset of serum disease.....	1			2	1	1	4	1			2																		
Anaphylactin first demonstrated in blood serum..									1	2		1	3*		1			1		1†		1		1					
Recovery from serum disease.											2	1	1		2	2				1						1	2		
First positive skin reaction..							1		1			2	2	2	2			1	1				1‡					1‡	1‡

The numerals indicate the number of cases.

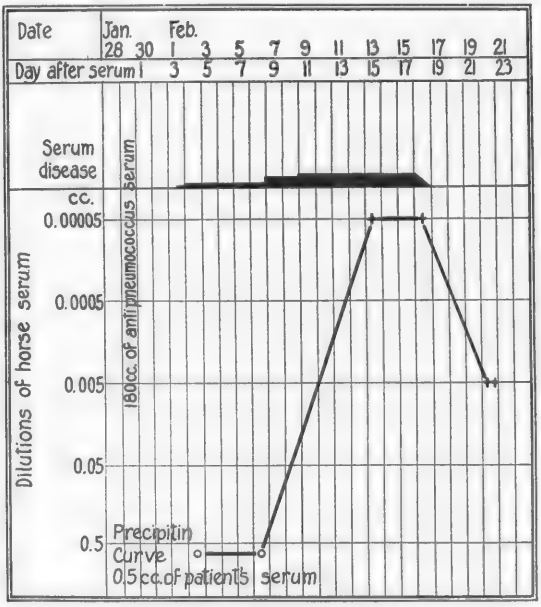
* One experiment doubtful but positive on 24th day.

† Doubtful reaction.

‡ No serum disease.

the average time relation between the appearance of anaphylactin and the course of serum disease. In Table III the average onset of serum disease came 4 to 8 days after the injection of serum, whereas anaphylactin makes its appearance 10 to 15 days after the injection of serum, and is more closely associated with the subsidence of serum sickness than with its onset.

Much the same relation was found to exist between the time of



TEXT-FIG. 2. The time relation of precipitin formation to serum disease. W. V. Type I pneumonia. 180 cc. of serum in two doses, January 29, 1917.

appearance of precipitin in the blood and the course of serum sickness in eleven cases that were studied. Precipitins were not demonstrable in the patient's blood serum until 3 or 4 days after the onset of serum sickness. After this time they rose rapidly in concentration to reach their maximum at the time of recovery. After recovery they diminished either rapidly or gradually in concentration. Text-fig. 2 is constructed from a typical protocol.

TABLE IV.

The Relation between the Onset and Subsidence of Serum Disease and the Appearance of Precipitins in the Circulation in Eleven Cases.

Days after injection of horse serum.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Onset of serum disease.....	1		1	2	2	1	1	1	1	1																							
Appearance of precipitins....									1	1	1	2	1	1	1	1		1	1														
Recovery from serum disease.		1										1	1		2		1		1	1	1					1							1

The numerals indicate the number of cases.

Precipitins were present in low concentration in the blood serum of three patients, in whom the serum sickness ran a protracted course, from the end of the 1st week to a period near the close of the disease when they rose rapidly in concentration. Wells and Weil have both made similar observations. On several occasions fever, which is often a late symptom of serum disease, was associated with the presence of precipitins in the circulation.

The composite Table IV, from the protocols of eleven cases, shows definitely the tendency for the appearance of precipitins in the circulation to precede immediately recovery from serum sickness.

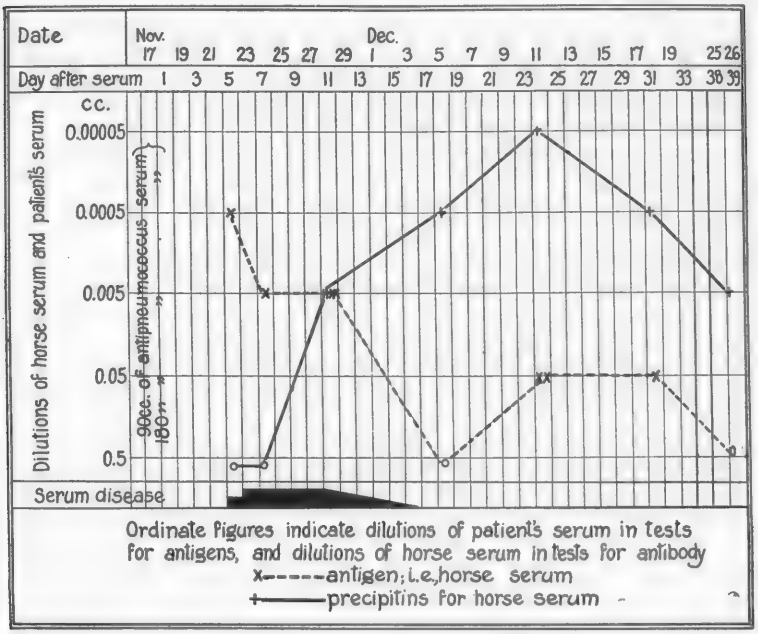
The relation of the appearance of the skin reaction to the course of serum disease in the cases that we have studied is made evident in Text-fig. 1. This reaction manifested itself only when serum disease was well advanced or had subsided, but since the skin became hypersensitive in all individuals who had received injections of horse serum and persisted over long periods of time, it seems probable that this reaction has a different significance from that of precipitin and anaphylactin formation, for the expulsion of these antibodies into the circulation is transient in character and definitely associated with recovery from serum disease, whereas the altered reaction of the skin is persistent and probably represents a changed characteristic of cells rather than a product of altered tissue reactions.

Our observations indicate a close relation between the appearance of antibodies for horse serum in the circulation and the termination of serum sickness. In no instance did antibodies appear either before or during the early stages of the disease, and in a few patients whose serum sickness was of unusually long duration there was a definite delay in the appearance of antibodies in the circulation. Further studies that have been made suggest that the appearance of antibodies in the circulation in great concentration is not only related to the termination of serum sickness but actually assists in bringing this about.

Early in the observations it became evident that circulating antibody to horse serum did not make its appearance unless it was preceded by a severe reaction in the tissue cells; namely, serum sickness. It seems highly improbable, therefore, that serum sickness depends upon a union of antigen and antibody in the circulation, but rather

that such a union, if it takes place, must do so, as Weil and others have frequently emphasized, within the cells of the body.

It is, however, obvious that antigen must be present either in the circulation or within the cells in order to bring about the reaction which we recognize as serum disease. Repeated observations have been made by us and by others to show that precipitinogen, or horse


Date
Nov.
Dec.
17 19 21 23 25 27 29 1 3 5 7 9 11 13 15 17 19 25 26
Day after serum
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 36 39
cc.
0.00005
0.0005
0.005
0.05
0.5
Dilutions of horse serum and patient's serum
90cc. of antipneumococcus serum
180cc.
Serum disease
Ordinate figures indicate dilutions of patient's serum in tests for antigens, and dilutions of horse serum in tests for antibody
x-----antigen; i.e., horse serum
+——precipitins for horse serum

TEXT-FIG. 3. Balance between antigen and precipitin in the circulation, and their relation to the course of serum disease. M. B., female; age 32 years. Type I pneumonia. Total 270 cc. of horse serum.

serum, remains in the circulation during at least a part of serum disease, so that both precipitin and precipitinogen are present in the circulation during serum sickness and for a few days thereafter. Most observers state that the antigen disappears more rapidly than precipitin, which remains in the circulation for days or weeks after recovery from serum disease. We have made an attempt to deter-

mine, by the methods of precipitation and the production of active anaphylaxis in guinea pigs, the exact time of disappearance of antigen from the circulation and the relation of its disappearance to the concentration of antibodies and to the duration of serum sickness. These observations, together with some others upon the absorption of antigen, will be reported later. Repeated observations have shown, as a rule, that there is a rapid disappearance of antigen upon the appearance of antibodies in the circulation in great concentration, and that the disappearance of antigen or the rapid diminution of antigen follows the rapid rise of precipitin and is coincident with recovery from serum disease. Text-fig. 3 is the reconstruction of a typical series of protocols showing the relation between precipitinogen, *i.e.* horse serum, and precipitin to the course of serum disease, and its termination.

DISCUSSION.

The experiments and observations recorded show that serum sickness is essentially dependent upon a reaction which takes place within the cells of the body and is probably dependent upon the formation of a toxic substance within these cells. Following this violent cellular reaction antibodies for horse serum are at first slowly and later rapidly extruded into the circulation in great concentration. As the antibodies rise in concentration the antigen rapidly disappears and coincident with this reversal in antibody-antigen content of the serum, serum sickness abates and the patient recovers. Should the antibody formation be slight in amount, the antigen may persist in large quantities in the circulation and the disease may be prolonged or may relapse.

We have shown that from the time of the primary injection of serum up to the onset of serum sickness, that is during the incubation period of this disease, the concentration of antigen in the circulation remains practically constant and antibodies to horse serum cannot be demonstrated. It seems highly probable, however, that during this period antisubstances are being formed within the cells and that at the time of onset of serum sickness, which is often explosive in character, there is a sudden union between the antibodies within the cells and circulating antigen which bathes the cells. The

course of serum disease depends at this time upon the production of antibody and the amount of available antigen. If antibody is formed in large amount and the quantity of antigen originally introduced is small, it is probable that the circulating antigen will rapidly be dispensed with and serum disease will be of short duration and mild in character. If, however, the amount of antigen originally injected is large and the formation of antibodies is slow and inefficient, it is highly probable that the available antigen will persist over long periods and that the serum disease will be severe in character and prolonged or relapsing in type. The production of circulating antibodies, therefore, seems a purely protective mechanism which is the method the body uses to destroy antigen, in this case horse serum. Since in serum disease the antigen is incapable of reproducing, the process can be accomplished, as a rule, rapidly and effectively, and recovery is prompt and complete.

Although we must always be cautious in drawing conclusions from analogies in biological experimentation, it is nevertheless admissible to suggest that these results may throw light on some of the methods by which the body rids itself of the injurious protein substances which represent antigen in an acute infectious disease.

CONCLUSIONS.

1. The injection of horse serum either in small or in large amounts in human beings is always followed sooner or later by the development of hypersensitiveness of the skin to subsequent injections of horse serum. For the development of this reaction serum disease is not essential.

2. The blood serum of most patients who suffer from an attack of serum disease following injections of horse serum shows anaphylactin and precipitin for horse serum.

3. Anaphylactin and precipitin cannot be demonstrated in the blood serum of patients treated with horse serum who do not later present symptoms of serum sickness.

4. The appearance of anaphylactin and precipitin precedes shortly recovery from the disease.

5. With the appearance in the serum of antibodies to horse serum in great concentration, the antigen rapidly diminishes or disappears.

6. It is probable that the extrusion of these antibodies into the circulation is the result and not the cause of serum sickness. Their presence serves to neutralize or destroy the antigen and thus determines the recovery from serum sickness.

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IMMUNITY IN INTESTINAL OBSTRUCTION.

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During the past few years a large amount of experimental work has been done in order to solve the problem of the cause of death in intestinal obstruction. This work has added to the knowledge of the physiology of the intestinal tract, but the cause of death from intestinal obstruction still remains unknown. It is generally conceded that a systemic bacterial invasion by the organisms of the obstructed intestine does not occur, and most of the clinical and experimental evidence so far obtained points to a quickly developing and rapidly fatal toxemia. The nature of the toxin is disputed, and investigations on this point thus far reported are not conclusive. The most extensive studies in this field have been made by Whipple, Stone, and Bernheim (1, 2, 3, 4), who ascribe the symptoms to a toxic primary proteose formed by the perverted activity of the intestinal mucosa. Leaving aside the question of how it is formed, we wished to determine, if possible, whether the toxic factor is really a primary proteose. A method of attack was suggested by the work done by Whipple, Stone, and Bernheim (3) in attempting to produce an immunity to the obstruction toxin. If an immunity to the toxin can be demonstrated it will show that the toxic factor in all probability belongs to that group of substances which have antigenic properties, namely the proteins, and their primary product of hydrolysis, the proteoses. The claim that a relation exists between antibody formation and non-proteins is apparently erroneous.

The conclusions of Whipple, Stone, and Bernheim,[1] as a result of their studies on immunity in intestinal obstruction, are not, in our opinion, warranted by the observations they have reported. Un-

[1] Whipple, Stone, and Bernheim (3), p. 164.

less extremely marked, immunity to a disease or toxin is always difficult to determine. In regard to the presence or absence of immunity in intestinal obstruction, we had noticed that dogs showed an extreme variability in their resistance to intestinal obstruction produced in various ways, and we believe that this normal variation recognized by Whipple and others accounts for most of their results.

The methods of Whipple, Stone, and Bernheim in localizing the immunity of a so called immunized dog should be noted. They obtained serum from a dog immunized by sublethal doses, added a lethal amount of duodenal fluid obtained from a closed duodenal loop, chloroform, and toluene, and incubated the mixture for 20 hours at 38°C. It was then tested, filtered, and injected in dogs. The injection caused death, and hence they concluded that there was no antiferment in the serum. Their further study was as follows:

"Many of the immune organs were washed free from blood and ground to a paste which was combined with a lethal dose of the duodenal loop fluid, diluted with water to a thin paste, and allowed to autolyze at 38°C. for 2 to 5 days with chloroform and toluol. The filtrate was then tested on normal dogs by intravenous injection. The spleen and lung emulsion destroyed the poison. The liver juice (Buchner press), diluted and filtered, also destroyed it rapidly. The intestinal mucosa destroyed some of the poison but a part remained even after 5 days' digestion. A fresh mixture of liver juice and loop poison gave fatal intoxication, showing that the reaction is not prompt or does not take place in the blood stream. This result serves as a control. If the protective action resides in a single type of cell, it is the endothelial cell that may be concerned, but it is of course possible that various body cells may develop the reaction or produce the ferment."

Can we conclude that an immunity reaction is the cause of the loss of toxicity by a fluid of complicated composition allowed to autolyze 5 days under toluene and chloroform with organ extracts? It may be true that the liver and spleen would contain more antibody or a higher concentration, were there any formed, than the blood serum, but there would certainly be some liberated into the blood, and it is not probable that the intestinal mucosa and lung would contain large amounts of a neutralizing substance and the blood none. The fact that "fresh mixture of liver juice and loop poison gave fatal intoxication, showing that the reaction is not prompt or does not take place in the blood stream" would indicate that the reaction is not that between an antigen and an antibody. The statements that they discovered no neutralizing principle in the serum of immunized dogs and that they observed nothing of an ana-

phylactic reaction in guinea pigs detract from the evidence that the fluid has antigenic properties.

Davis and Morgan (5) were not able to confirm the results of Whipple and others, using autolysates of normal cat organs and cat serum with the fluid from dog closed loops, since they found that cats were apparently more highly resistant or immune to the intoxication of intestinal obstruction than dogs.

Nesbitt (6) endeavored to show that neurine may be split off from the choline in lecithin and is present in the intestinal canal during obstruction. He also noticed a ptomaine (unidentified) as a constituent of the material above an obstruction. Barger and Dale (7) demonstrated the presence of the depressor substance β-iminazolylethylamine in the intestinal mucosa. Mellanby and Twort (8) corroborated this and isolated a bacillus which they claimed could convert histidine into this substance. Murphy and Brooks (9) observed:

"5. The toxicity of the loop content is not destroyed by heating to 60°C. until sterile, or even by boiling.

6. The toxicity of the fluid is very much decreased by filtration through a Berkefeld filter, so that to produce death a dose of filtrate corresponding to several times the lethal dose of unfiltered fluid is necessary.

7. The amount of filterable toxin is increased by prolonged autolysis."

Draper (10) was unable to find a proteose in the 1,000 cc. of loop fluid which he analyzed. These results all indicate that the toxic factor is not of a protein nature.

Methods.

As the symptoms caused by a closed intestinal loop, whether produced by ligature and a reconstruction of the gastrointestinal tract by gastroenterostomy, or by resection of the loop and an end to end anastomosis of the intestine, parallel closely the symptoms of acute intestinal obstruction, we have used the following control procedures: (1) the production of open intestinal loops, (2) the antemortem removal of closed intestinal loops, (3) the production of a blind duodenal stump, (4) ligature of the duodenum with no anastomosis, (5) injection of the material from closed intestinal loops.

Dogs were used in all the experiments. All operations were done under complete surgical anesthesia (morphine-ether) with the usual aseptic technique.

1. Production of Open Intestinal Loops.—As shown by Dragstedt, Moorhead, and Burcky (11), a certain proportion of dogs, in their work 50 per cent, can survive an open unwashed loop of the duo-

denum which is permitted to drain into the peritoneal cavity. This has been confirmed by the writers in a large number of dogs, and open loops have been made of the duodenum, jejunum, and ileum. While the number of dogs operated on in the lower part of the small intestine is not large, the work indicates that the lower the loop is, the smaller is the percentage of animals surviving an open unwashed intestinal loop—in approximate proportion to the increased number of bacteria found. The open loops upon later examination are in about half the instances found open and draining, while in the rest they are tightly closed by omental adhesions and are usually found fairly distended with a thick yellowish white material, which is often not sterile. Where the loops have been found open and draining a marked edematous and hemorrhagic appearance of the omentum and serous surfaces of the viscera is noticed, and three dogs died about 3 weeks after the operation from omental hemorrhage.

Whipple and his associates (4) produced an open loop of a different sort. The duodenum just below the pancreatic duct was cut across and ligated with inversion of ends and closure. A gastroenterostomy was done just below the duodenojejunal flexure so that a loop of the duodenum was produced which could drain into the jejunum. They state:

"The presence of such a partially isolated duodenal loop may be associated with intoxication, more or less severe, which will bring about an immunity reaction in the body cells. The intestinal mucosa from such a dog has the characteristic property of immune tissue; it can destroy with some rapidity the duodenal loop fluid *in vitro* and render the mixture harmless when given intravenously to a normal dog."

If there is a specific toxic secretion in a loop of such a nature that it can act as an antigen upon absorption, it is logical to conclude that the constant absorption of this substance would render dogs surviving open intestinal loops highly immune to the toxins of intestinal obstruction. All our methods of testing resistance to intestinal obstruction were tried out on these dogs, with the result given in Tables I, II, and X.

2. Antemortem Removal of Closed Intestinal Loops.—To answer the objection that an open intestinal loop is not an obstructed loop and hence the conditions necessary for the secretion of a hypothetical toxic proteose do not prevail, resected and closed unwashed loops of the beginning jejunum were produced in dogs and removed before the

loop had perforated, but as long after the production of the loop as the condition of the animal would warrant. The majority of the loops were markedly distended, already cyanotic in color, and contained approximately 80 to 110 cc. of bloody fluid. Although the period of immunization is necessarily short, it is to be expected that animals almost moribund from acute intestinal obstruction would, upon recovery, show a marked immunity if it can be produced. Here, too, there is tissue destruction, with resultant absorption of protein split products, and the chance of an increased absorption of the hypothetical toxic proteose. Hartwell, Hoguet, and Beekman (12) say that the toxemia is in proportion to the tissue necrosis, and without the latter there are no toxic symptoms. About half the dogs whose loops were removed before perforation died, indicating that there had been a marked absorption of toxic material by the time of the operation. Removal of the loop is a short and simple procedure. It was tried as an immunizing method in three series of dogs (Tables VI, IX, and X).

3. Production of a Blind Duodenal Stump.—Early in the work the authors noted that dogs in which a drained loop of the duodenum was made and the reconstruction of the canal effected by gastroenterostomy were not in as good condition as dogs in which end to end anastomosis was made. This was noted by Sweet, Peet, and Hendrix (13), and Whipple, Cooke, and Stearns (14) later operated to produce a blind duodenal stump as a method of causing a chronic type of obstruction in dogs. Most of the dogs in their series died in from 1 to 3 weeks with symptoms similar to those of obstruction. According to Whipple, these dogs have a definite tolerance to proteose injections. In conjunction with some other work the authors made a number of blind duodenal stumps, making the gastroenterostomy at the greater curvature and as near the pylorus as practicable, varying the length of the blind stump from about 8 to 50 cm.

If this type of operation results in a chronic obstruction and the toxin thereof is identical with the toxin of acute obstruction, this will be a better procedure to test out the immunity of a dog than the production of a closed loop as there is no perforation peritonitis to obscure results.

4. Ligature of the Duodenum with No Anastomosis.—In view of the

fact that the average length of life for closed loop dogs was higher in Whipple's series than in ours, a comparison of methods was necessary. The difference is easily explained. The closed loops as made by Whipple were produced by double ligature of the duodenum, just below the lower pancreatic duct and again at the duodenojejunal junction, reconstructing the tract by gastroenterostomy. This, of course, results in a longer loop than is possible by the method of resection and an intestinal anastomosis. The mechanical feature of this will, of course, explain the more rapid swelling of the smaller loop and an earlier death. Then there is the other factor, the cutting through of the ligature at either end of the loop by Whipple, permitting an escape of fluid from the loop, decreasing the tension within the loop, and thus preventing such a rapid swelling with consequent occlusion of the blood supply and necrosis of the intestinal wall as is found in the resected loop. This phase of the ligatured loop was studied extensively by the authors. The method consisted in ligating the duodenum with a single ligature, the size of which varied, and making no reconstruction of the canal, thus leaving the animal with an uncomplicated high obstruction. The ligature was buried with Lembert stitches. It was found that with a fairly heavy linen ligature the cutting through of the tissues and the restoration of the lumen begins in about 48 hours in the majority of instances, there being a lumen of about 1 to 2 mm. upon the 3rd day. Wide variations have been found. One dog showed a lumen of but 1 mm. after 11 days, while some showed two-thirds normal lumen after 48 hours. About 50 per cent of the dogs in which a ligature of the duodenum was done recovered completely. These dogs were tested for an immunity that they might have acquired as a result of the condition of acute obstruction which they had endured for about 48 to 72 hours.

5. Injection of the Material from Closed Intestinal Loops.—The last method of immunization was that used by Whipple; namely, the intravenous injection of the fluid from closed intestinal loops. The fluid was prepared according to the method of Whipple, with the exception that it was used within 2 or 3 days after preparation.

It is well known that many non-toxic substances, when kept in contact with such material as toluene and chloroform, may acquire a

certain degree of toxicity, and, aside from this, putrefaction is by no means prevented and any number of toxic substances may be formed which were not in the fluid at the time of collection. We were surprised to note the extreme variation in toxicity of different samples of fluid collected. 10 cc. in many instances have been fatal, while as much as 115 cc. of the undiluted fluid have caused no marked symptoms in other cases. Dogs that recovered from injections of this fluid were tested in the various ways outlined below for the existence of immunity or increased tolerance to intestinal obstruction.

Methods of Studying Immunity to or Tolerance to Intestinal Obstruction.

Production of Closed Intestinal Loops.—This is always a questionable procedure, owing to the fact that many dogs die from perforative peritonitis, to which, of course, immunity is impossible. The closed loops were made in the duodenum, as a fair proportion of the dogs die before the loop has perforated and in these dogs increased resistance can be readily observed. A dog in which the cause of death is perforative peritonitis can be easily observed after the production of the loop and the degree of resistance of the dog, in the earlier stages before the loop has perforated, noted.

Closed intestinal loops were made as a test procedure (*a*) in dogs which were strong and healthy after the open loop operation (Table I), (*b*) in dogs which had recovered from a ligatured obstruction (Table III), (*c*) in dogs from which closed loops had been removed (Table VI), and (*d*) in dogs which had previously been injected with closed loop fluid (Table VII).

Blind Duodenal Stump.—The question and method of production of a blind duodenal stump has been discussed above. Here there is no complication such as perforation and hence the results should be clear and indicative.

Blind duodenal stumps were produced in order to test the resistance (*a*) of dogs which had previously had open loops (Table II), (*b*) of dogs which had recovered from a ligatured obstruction (Table IV), (*c*) of dogs which had previously been injected with closed loop fluid (Table V), and (*d*) of a dog from which a closed loop had been removed (Table IX).

Injection of Closed Loop Fluid.—If the fluid contains the obstruction

toxin, then dogs recovered from obstruction should be more resistant to injections of it than normal dogs. On the other hand, if the fluid contains other toxic substances than that to which the dog may be expected to have increased resistance, there will be no immunity observed, following injection of the fluid, although the animal may in reality be immunized to the obstruction toxin. If, however, the other toxic substances present, as well as the obstruction toxin, are of such a nature that they can act as antigens, it is to be expected that injection of the fluid will markedly increase the resistance of the animal to the fluid. This should be true to an observable extent even if none of the toxic substances present besides the obstruction toxin have antigenic properties.

Is it not as logical to assume that if a number of toxic substances are found in the intestinal loop fluid the cause of death is due to all of them as it is to ascribe it to a particular perverted secretion, because the possible chemical nature of one toxin has been defined?

The resistance of dogs which had recovered from a ligatured obstruction, of dogs from which closed intestinal loops had been removed, of a dog which survived a closed washed intestinal loop (washing with sterile water and ether), of dogs with open intestinal loops, and of dogs which had received previous injections of loop fluid, to the injection of closed loop fluid, was compared with the resistance of normal dogs. The results are summarized in Table X. The results of injection of loop fluid into normal dogs is also included in this table to demonstrate the extreme variation in resistance that is met with normally.

Ligature of the Duodenum.—Although the percentage of recovery in normal dogs is comparatively high (about 50 per cent), it is to be expected that dogs immunized by any means to the toxin of obstruction would have such a resistance to the toxin that they would survive until the obstruction was relieved, by the cutting through of the ligature, to a much greater extent and in a greater number of cases than normal dogs. The percentage of recovery should be markedly increased. Our data on this point are not extensive for percentage results, but they are indicative.

Ligature of the duodenum was done as a test procedure only on a series of dogs which had received injections of closed loop fluid

(Table VIII). Four dogs of the seven survived over 5 weeks, which gives practically the same percentage of survival as in the control dogs given below.

Control Dogs.

Ligature of the Duodenum.—The duodenum just below the lower pancreatic duct was ligated in thirty-nine dogs with a linen ligature buried by Lembert stitches. In twenty-one dogs there was complete recovery. Seven of the remaining animals died in from 4 to 10 days from pneumonia contracted as a result of the toxemia and decreased resistance from the obstruction. The remaining eleven died in less than 96 hours with an uncomplicated autopsy picture.

Blind Duodenal Stump.—A blind duodenal stump was made in seventeen dogs by cutting the duodenum and in some instances the beginning of the jejunum, infolding the proximal end, and anastomosing the distal segment to the greater curvature of the stomach as close to the pylorus as practicable. The length of the blind stump varied from 10 to 65 cm. Two dogs are still living (3 months) and show no toxic symptoms. One died at the end of 2 months in extreme cachexia, the rest surviving the operation from 3 to 25 days. We found no direct correlation between the length of the blind stump and the degree of toxicity.

Closed Duodenal Loops.—The dogs in this series (twenty-six dogs) all died in from 24 to 96 hours, the average length of life being 48 hours. Nineteen of the loops were found to be perforated at autopsy. This gives a percentage of 73 dying from perforative peritonitis. If there is an increased tolerance in immune dogs, this percentage should be markedly increased and nearly all dogs would die as a result of the perforative peritonitis before the uncomplicated obstruction toxemia should prove fatal. None of the control dogs survived this type of loop in our series, although Sweet, Peet, and Hendrix report several instances in which the animal has lived for weeks. In the work of Dragstedt, Moorhead, and Burcky only two loops in six were found to be perforated. This gives a percentage of 33, but percentages from such a small series are misleading. Out of seven closed duodenal loops washed with water and ether, they found four perforated at autopsy.

TABLE I.

Open Loops with Later Production of Closed Loops.

Dog No.	Location of open loop.	Condition of open loop at 2nd operation.	Interval.	Location of closed loop.	Degree of toxemia preceding death.	Length of life.	Perforation.
			days			*hrs.*	
1	Duodenum.	Open.	36	Jejunum.	Toxemia after 24 hrs.	48	−*
2	"	Closed.	100	"	" " 15 "	19	+
3	"	Open.	33	"	" from start.	36	−
4	"	Closed.	21	"	" " "	60	−
5	"	Open.	26	"	Active until last few hours.	90	+
6	"	"	26	"	" " "	46	+
7	Jejunum.	"	62	Duodenum.	" " "	42	+
8	"	"	17	"	Toxemia from start.	40	+
9	"	"	24	"	" " "	26	+

* − indicates no perforation; +, perforation.

TABLE II.

Open Loops with Later Production of a Blind Duodenal Stump.

Dog No.	Location of open loop.	Condition of open loop at 2nd operation.	Interval.	Distance of blind end from pylorus.	Symptoms and remarks.	Length of life.
			days	*cm.*		
10	Jejunum.		16	38	No signs of toxemia.	Living (3 mos.).
11	"	Open.	16	42	Good recovery, then gradual decline.	7 days.
12	"		16	36	Cause of death unknown.	24 hrs.
13	"	Open.	8	45	Gradual decline.	5 days.
14	"	"	21	42	" "	5 "

TABLE III.

Dogs Recovered from a Ligatured Obstruction with Later Production of a Closed Loop.

Dog No.	Location of ligature.	Interval.	Location of closed loop.	Symptoms and remarks.	Length of life.	Perforation.
		days				
15	Lower pancreatic duct.	19	Duodenum.	Toxemia and recovery.	Living (3 mos.).	
16	" "	9	"	No toxic symptoms.	" (3 ").	
17	" "	10	"	Toxemia from start.	39 hrs.	+
18	" "	10	"	" " "	29 "	+
19	" "	9	"	" " "	32 "	+
20	Lower end of duodenum.	13	"	" " "	34 "	+
21	" "	13	"	Active until 4 hrs. before death.	56 "	+
22	" "	14	"	" " "	34 "	+

TABLE IV.

Dogs Recovered from a Ligatured Obstruction with Later Production of a Blind Duodenal Stump.

Dog No.	Location of ligature.	Interval.	Length of blind stump.	Symptoms and remarks.	Length of life.
		days	*cm.*		
23	Duodenum.	12	28	Cachexia and malnutrition.	25 days.
24	"	12	34	Pneumonia.	36 hrs.
25	"	10	38	Cachexia and malnutrition.	10 days.
26	"	9	30	" " "	20 "

TABLE V.

Dogs Injected with Closed Loop Fluid with Later Production of a Blind Duodenal Stump.

Dog No.	Times injected.	Interval.	Length of blind stump.	Symptoms and remarks.	Length of life
		days	*cm.*		
27	1	18	12	Gradual cachexia.	8 days.
28	2	9	35	" "	4 "
29	1	17	25	Lively.	Living (3 mos.).
30	1	17	36	Gradual cachexia.	5 days.

TABLE VI.

Closed Loops Removed and Second Closed Loops Made.

Dog No.	Location of 1st loop.	Interval before removal.	Condition at removal.	Interval.	Location of 2nd loop.	Symptoms and remarks.	Length of life.	Perforation.
		hrs.		*days*			*hrs.*	
31	Jejunum.	30	$\frac{4}{4}$*	28	Duodenum.	Toxemia from start.	34	+
32	"	21	$\frac{2}{4}$	19	Jejunum.	Mild toxemia from start.	48	+
33	"	20	$\frac{1}{4}$	15	Duodenum.	Active till 2 hrs. before death.	36	+
34	"	34	$\frac{3}{4}$	13	"	Toxemia from start.	30	+
35	"	72	$\frac{3}{4}$	7	"	Mild toxemia throughout.	38	−
36	"	48	$\frac{3}{4}$	6	"	Sudden onset of toxic symptoms 2 hrs. prior to death.	26	+
37	"	$34\frac{1}{2}$	$\frac{2}{4}$	40	"	Toxemia continuous.	48	−

*$\frac{4}{4}$ indicates distention to point of perforation; $\frac{1}{4}$ indicates beginning distention, etc.

TABLE VII.

Dogs Injected with Closed Loop Fluid with Later Production of Closed Loops.

Dog No.	Times injected.	Interval.	Location of closed loop.	Symptoms and remarks.	Length of life.	Perforation.
		days			*hrs.*	
38	1	27	Duodenum.	Mild toxemia.	36	+
39	1	12	"	" "	50	+
40	2	12	"	Active until last few hours.	38	+
41	1	8	"	" "	42	+
42	1	8	"	Toxemia from start.	28	−
37	1	12	"	" continuous.	48	−
43	1	7	Jejunum.	" "	24	−

TABLE VIII.

Dogs Injected with Closed Loop Fluid with Later Ligature of the Duodenum.

Dog No.	Times injected.	Interval.	Location of ligature.	Symptoms and remarks.	Length of life.
		days			*days*
44	3	7	Lower pancreatic duct.	Gradual toxemia.	7
45	3	7	" " "	" "	9
46	2	9	" " "	" "	5
47	1	20	" " "	Beginning toxemia after 24 hrs.	36
48	2	3	" " "	Complete recovery.	
49	1	4	" " "	Toxemia after 24 hrs.	48
50	1	5	" " "	Complete recovery.	

TABLE IX.

Closed Loop Removed with Later Production of a Blind Duodenal Stump.

Dog No.	Location of loop.	Condition at removal.	Interval.	Length of blind stump.	Symptoms and remarks.	Length of life.
			days	*cm.*		*days*
30	Jejunum.	¾ distended.	34	36	Toxemia after 2nd day.	5

TABLE X.

Comparative Resistance of Dogs to the Injection of Closed Loop Fluid.

Fluid.	Dog No.	Condition of animal.	Weight.	Amount injected.	Amount per kilo.	Result.
			kg.	*cc.*	*cc.*	
W. R.	43	Normal.	6	6	1	Toxemia and recovery.
"	51	"	11	24	2.2	" " "
"	52	"	8.3	24	2.9	Dead in 5 hrs.
"	53	"	5.5	24	4.3	" " 8 "
"	16	Ligature and closed loop.	8	16	2	" " 18 "
A_2	29	Normal.	8	40	5	Good recovery.
A_2	54	"	8	80	10	Toxemia and recovery.
A_2	55	"	8	105	13	Dead in 10 hrs.
A_2	56	Closed loop removed.	6	33	5.5	Good recovery.
A_2	30	" " "	10	120	12	" "
A_2	57	" " "	10.5	105	10	Toxemia and recovery.
404	46	Normal.	9.4	28	3	Good recovery.
404	58	"	11	33	3	" "
404	38	"	8.9	27	3	Marked toxemia and recovery.
404	27	"	9	54	6	Good recovery.
404	59	Open loop.	6.3	19	3	" "
404	44	Two previous injections.	10.4	31	3	" "
404	45	" " "	12.5	38	3	Toxemia and recovery.
404	60	Open loop.	10.4	31	3	Marked toxemia and recovery.
404	46	One previous injection.	9.4	50	5.3	" " "
404	61	" " "	11	60	5.5	Dead in 6 hrs.
439	49	Normal.	7.7	45	5.8	Good recovery.
439	50	"	13	100	7.7	Toxemia and recovery.
439	48	One previous injection.	7.3	49	6.7	" " "
439	62	" " "	6	60	10	Dead in 12 hrs.
XO2	47	Normal.	13.2	33	2.5	Good recovery.
XO2	63	"	6.4	32	5	" "
XO2	64	"	7.3	50	6.7	Dead in 8 hrs.
XO2	65	"	5.7	17.1	3	" " 8 "
XO2	66	"	13	98	7.5	" " 6 "
XO2	67	Open loop and closed washed loop.	8.6	27	3.1	Toxemia and recovery.
XO2	68	Open loop.	14.2	100	7	Good recovery.
XO2	69	One previous injection.	7	50	7.1	Dead in 4 hrs.
XO2	37	Closed loop removed.	10.7	32	3	Toxemia and recovery.
658	39	Normal.	17	102	6	Good recovery.
658	40	"	12	72	6	Toxemia and recovery
658	28	"	5	45	9	Good recovery.
27	70	Normal.	8	6	0.8	Dead in 19 hrs.
27	44	"	10.4	15	1.4	Toxemia and recovery.
27	45	"	12.5	5	0.4	" " "

DISCUSSION.

With few exceptions immunized dogs showed no greater resistance to subsequent obstruction than normal dogs, and in many instances they showed less. Two dogs that recovered from a ligation of the duodenum survived a closed unwashed duodenal loop, and are still living after 3 months. Upon later examination these loops were found to be only moderately distended and of good color. So far we have had no normal dogs survive a closed duodenal loop to this extent, but we are inclined to believe that the previous obstruction has altered the secretion-absorption ratio so that upon production of a closed loop there was no distention with consequent tissue necrosis, inasmuch as Sweet, Peet, and Hendrix report several instances of a normal dog surviving closed loops, and Dragstedt, Moorhead, and Burcky have shown that dogs can survive closed loops washed with ether. We do not consider that any immunity is shown by these cases. One dog immunized by injection survived a blind duodenal stump indefinitely. Controls have done this, however, and this is, therefore, no indication of an increased resistance.

Of the twenty-nine closed loops produced in immune dogs, twenty-one were found to be perforated after death. This gives a percentage of 72 for dogs dying of perforative peritonitis, which is no higher than that in control dogs and indicates that the immune dogs have no greater resistance to the toxemia than control dogs.

There still remains the possibility of an increased tolerance to the poison of intestinal obstruction. It is well known that carrion-eating animals can ingest quantities of putrefying protein that would poison man. What is the nature of the resistance to the poisons, and is it possible that dogs recovered from intestinal obstruction might show a slight increased tolerance to a later similar condition?

Our experiments so far do not indicate an increased tolerance, but if there is a tolerance of slight grade, it would take a great many experiments to demonstrate it. If this should prove to be the case, we believe that our work warrants the statement that the increased tolerance is due to some variable factor, such as diminished absorption in that section of intestine which was affected by the obstruction, since an increased tolerance has not been noticeable in a great number of our experiments.

CONCLUSIONS.

1. There is no increased immunity or tolerance to intestinal obstruction after recovery from previous obstruction.

2. Dogs recovered from intestinal obstruction are not more resistant to injections of closed loop fluid than normal dogs.

3. Dogs injected with closed loop fluid are not more resistant to intestinal obstruction than normal dogs.

4. In dogs the normal variation in resistance both to intestinal obstruction and to the injection of closed loop fluid is large.

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THE ACTION OF ANTISEPTICS ON THE TOXIN OF BACILLUS WELCHII.

A Preliminary Note.

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The chemical sterilization of wounds has resulted in much work on the bactericidal action of the antiseptics in general use.[1,2] In a former paper we reported[3] on the solvent action of some of the chlorinated antiseptics on necrotic tissue, pus, blood clot, and plasma clot. The only evidence that these antiseptics may have a destructive action on bacterial toxins is, first, the clinical observations of Carrel and Dehelly,[4] who noted that in patients with infected wounds treated with hypochlorite there seemed to be an amelioration of the general symptoms which they thought might be due to a reduction in the amount of toxin absorbed, and, second, some experiments by Lumière,[5] who found that pus containing virulent organisms, *B. tetani*, *B. welchii*, streptococci, and staphylococci, and presumably also bacterial toxins, became innocuous after the addition of hypochlorite solution and did not cause symptoms or death in animals injected with the mixture. Control animals, injected with the untreated pus, showed typical lesions. A second series of animals was injected with candle-filtered specimens of pus before and after treatment with hypochlorite solution. The animals that received filtrate from untreated pus showed symptoms of toxemia, while those in which the filtrate from the pus-hypochlorite mixture was injected showed no toxic effect.

It seemed desirable to perform a series of experiments with a definite toxin which could be quantitatively measured and a suitable, susceptible animal as an indicator. Bull and Pritchett[6] have demonstrated

[1] Dakin, H. D., Cohen, J. B., and Kenyon, J., *Brit. Med. J.*, 1916, i, 160. Dakin, H. D., and Dunham, E. K., *ibid.*, 1917, ii, 641.

[2] Dakin, H. D., Cohen, J. B., Daufresne, M., and Kenyon, J., *Proc. Roy. Soc. London, Series B*, 1916, lxxxix, 232.

[3] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155.

[4] Carrel, A., and Dehelly, G., The treatment of infected wounds, New York, 1917, 31.

[5] Lumière, A., *Compt. rend. Acad.*, 1916, i, 365.

[6] Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 119.

a toxin for *Bacillus welchii* which fulfills all the requirements of the so called group of soluble or exotoxins. They have standardized the virulence of this toxin, and confirmed the unpublished observation of Flexner[7] that the pigeon is highly susceptible to the toxin and that the lesions produced in this animal are similar to those observed in human cases of gas gangrene. As wounds infected with *Bacillus welchii* are frequently encountered in military surgery today, and as the antiseptics studied are used extensively on wounds of this character, it was decided to use the toxin of Bull and Pritchett and the pigeon as a very sensitive indicator of the relative toxicity of the various toxin-antiseptic mixtures in the series of experiments recorded here. Ten experiments were performed. Comparable results were obtained in all, and the three series recorded below are in every way typical.

Method.

Production of Toxin.—Virulent strains of *Bacillus welchii* were grown for 18 hours in the culture medium described by Bull and Pritchett,[6] which, briefly, is made as follows: To 10 cc. of 0.2 per cent glucose broth are added a few fragments of sterile rabbit muscle. Inoculations are made into this medium under a layer of sterile paraffin oil and the cultures incubated in a vacuum jar from which the air has been exhausted. After incubation, the fluid is centrifuged for 20 minutes at high speed and filtered through a Berkefeld N candle. The different lots of toxin produced in this way are found to differ considerably in potency. For example, 0.3 cc. of the filtrate used in Experiment 1 contained one fatal dose of toxin, while it required 1 cc. of the filtrate used in Experiments 2 and 3 to produce a similar effect. In all cases the toxic filtrate was titrated previously to its use to determine the smallest amount which would kill, in 12 hours or less, a pigeon weighing from 300 to 400 gm., and this amount was considered as one lethal dose.

Treatment of Toxin with Antiseptic.—Volumes of filtrate containing the required number of fatal doses were measured into Esmarch dishes. Horse serum, inactivated at 58°C. for 1 hour, was next added to the solutions in which it was used. Sodium chloride solution,

[7] Flexner, S., quoted by Bull and Pritchett.[6]

0.9 per cent, was added to the portions requiring additional volume, and the antiseptic to be tested was added last of all. The volume used for injection was kept constant in each experiment, with the exceptions noted below. The antiseptic was allowed to remain for 5 minutes in contact with the other substances to be injected, and then the entire volume was injected into the pectoral muscles of a pigeon. Before injection, the feathers were removed from the breast and the skin was washed with alcohol. The results of Experiment 1 are shown in Table I.

TABLE I.

Experiment 1.

Pigeon No	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result
	gm.			*cc.*	*cc.*	
1	410	1			3	Died in 12 hrs.
2	425	2	3 cc. of Dakin's solution. *			Lived.
3	425	4	3 " " " " *			"
4	425	4	3 " " " " *	1.5		"
5	410	2	3 " " phenol " †			Died in 10 hrs.
6	425	4	3 " " " " †			" " 5 "
7	240		3 " " Dakin's " *			Lived.
8	325		3 " " phenol " †			"

* Dakin's solution titrated 0.5 per cent sodium hypochlorite concentration (made from bleaching powder).

† Phenol solution, 0.25 per cent.

Experiment 1.—One fatal dose of toxin killed Pigeon 1 in 12 hours. 3 cc. of Dakin's hypochlorite solution, titrating 0.5 per cent sodium hypochlorite, protected Pigeon 2 against two fatal doses, and the same amount of the solution protected Pigeon 3 against four fatal doses of toxin. That blood serum will cause hypochlorite solution to decompose is well known, and that it will reduce the effectiveness of this solution, at least as a germicide, is shown by the experiments reported by Dakin and his coworkers.[1, 2] 3 cc. of Dakin's hypochlorite solution protected Pigeon 4 against four fatal doses of toxin, even in the presence of 1.5 cc. of horse serum. Phenol did not exhibit

any protective action. Pigeon 5, injected with a mixture of 3 cc. of 0.25 per cent phenol and two fatal doses of toxin, died in 10 hours. Pigeon 6, receiving the same amount of phenol solution but four fatal doses of toxin, died 5 hours after inoculation. Pigeons 7 and 8, injected with the antiseptics in the same amount and concentration as employed in the toxin-antiseptic mixtures injected into the other pigeons, survived, thus demonstrating that the antiseptics themselves were not toxic and could not have explained the death of Pigeon 5 in 10 hours and that of Pigeon 6 in 5 hours.

Experiment 2.—The results of this experiment, recorded in Table II, confirm those obtained in Experiment 1. Because the toxin available at this time was not so potent as that used in the first experiment, it was necessary to use greater quantities for injection. In order that the total volume of the solutions to be injected should not be increased above 12 cc. and that the relative concentration of the antiseptics should be of a degree comparable with those used in Experiment 1, it was necessary to concentrate them somewhat; therefore a triple strength Dakin's hypochlorite solution, titrating 1.5 per cent sodium hypochlorite concentration, and a 1 per cent phenol solution were used. The final strength of the sodium hypochlorite and of phenol in the injected mixtures was comparable with that of those of Experiment 1, inasmuch as the dilution was considerably greater. The results were the same as those recorded for Experiment 1 in Table I.

Experiment 3.—In this experiment the action of chloramine-T was contrasted with that of Dakin's hypochlorite solution and of phenol. The results shown in Table III were similar to those obtained when the hypochlorite solution was used and confirm those summarized in Tables I and II. They also show that chloramine-T is able to protect pigeons against at least three fatal doses of the toxin and that its action is still demonstrable when serum is previously mixed with the toxin and the antiseptic is required to act on it as well as on the toxin.

TABLE II.

Experiment 2.

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result.
	gm.			*cc.*	*cc.*	
9	270	1			7	Died in 15 hrs.*
10	310	3	2 cc. of Dakin's solution. †		3	Lived.
11	310	6	2 " " " " †			"
12‡	310	6	3 " " " " †	3		"
13	275	3	2 " " phenol " §		3	Died in 15 hrs.*
14	200	6	2 " " " " §			" "15 " *
15	240		2 " " Dakin's " †		6	Lived.
16	410		2 " " phenol " §		6	"

* Over night.

† Dakin's solution titrating 1.5 per cent sodium hypochlorite (triple strength).

‡ In this pigeon the volume injected was 12 cc., in the others 8 cc.

§ 1 per cent.

TABLE III.

Experiment 3.

Pigeon No.	Weight.	Fatal doses of toxin.	Antiseptic.	Horse serum.	0.9 per cent sodium chloride solution.	Result.
	gm.			*cc.*	*cc.*	
17	480	1			7	Died in 12 hrs.
18	470	3	5 cc. of Dakin's solution.*			Lived.
19	430	6	2 " " " " †			"
20‡	460	6	2 " " " " †	4		"
21	420	3	5 " " phenol " §			Died in 5 hrs.
22	500	3	5 " " chloramine-T " ‖			Lived.
23‡	500	3	5 " " " " ‖	4		"
24	320		5 " " Dakin's " †		3	"
25	450		5 " " phenol " §		3	"
26	310		5 " " chloramine-T " ‖			"

* 0.5 per cent sodium hypochlorite titration.

† 1.73 per cent sodium hypochlorite titration.

‡ In this pigeon 12 cc. were injected, in the others 8 cc.

§ 0.25 per cent.

‖ 2 per cent chloramine-T (equivalent to 0.5 per cent sodium hypochlorite).

DISCUSSION.

From the experiments outlined above it is apparent that Dakin's hypochlorite and chloramine-T solutions will destroy the toxin produced by *Bacillus welchii*. It has seemed more precise, for experimental purposes, to make the mixtures of toxin and antiseptic *in vitro*, but from the experiments of Lumière[5] and the clinical observations of Carrel and Dehelly[4] it seems possible that these solutions may exert a similar influence when used in the treatment of infected wounds. The fact that the detoxicating action was still demonstrable when the toxin was treated with serum before the addition of the antiseptic adds to the clinical significance of these observations, because the conditions then closely simulate those encountered when the antiseptic is applied to wounds.

Phenol solutions of a final concentration of 0.25 per cent exhibited no destructive action on the toxin, and all the animals injected with a toxin-phenol mixture succumbed in the 24 hour interval following inoculation.

The control pigeons (Nos. 7 and 8, Table I; Nos. 15 and 16, Table II; and Nos. 24, 25, and 26, Table III) always survived; the antiseptic substances in the quantities and concentrations used, therefore, were not of themselves lethal.

No attempt was made to determine the maximum number of fatal doses of toxin against which a given amount and concentration of antiseptic was able to protect, nor did we go into the question of the length of time that the antiseptic and toxin must be in contact before injection in order that detoxication may occur.

The pathology of the lesions in the pigeons that died in the above experiments was substantially the same as that described by Bull and Pritchett.[6] In those that did not die varying grades of local edema, congestion, swelling, and discoloration of the skin and subcutaneous tissue were observed. These lesions were never marked and in no instance did they approach those observed in the birds receiving injections which resulted fatally. Those receiving phenol alone showed slightly more marked lesions than those receiving the hypochlorite alone.

In no instance did the pigeons recorded "Lived" in the tables die in the interval of observation, which was at least 1 week and in most

instances 2 weeks or longer. Any possibility of retarded deleterious effects, therefore, from the toxin-antiseptic mixtures injected, is practically excluded.

Finally, it seems desirable to add that these observations are not recorded with the purpose of advocating the use of an antiseptic in the place of the specific antitoxin produced by Bull and Pritchett.[6] In human surgery the antiseptic treatment of infected wounds will doubtless be combined with specific serum therapy.[8]

CONCLUSIONS.

1. Dakin's hypochlorite and chloramine-T solutions will protect pigeons against multiple fatal doses of the toxin of *Bacillus welchii* when the antiseptic and the toxin are mixed *in vitro* and allowed to stand in contact for 5 minutes before injection.

2. The detoxicating action of the solutions is demonstrable also in the presence of serum.

3. Phenol solution, 0.25 per cent, has no such action.

We take this opportunity to thank Dr. Bull and Miss Pritchett for their help and advice in the production and use of the toxin.

[8] A comparison of the behavior of these antiseptics enables us to distinguish two groups. In one, the antiseptic while bactericidal possesses little or no destructive action upon the products of bacterial activity; of this group phenol is an example. In the other group, the antiseptic attacks chemically not only the bacteria but also their products and by an alteration or disintegration of the molecules of the latter alters their properties and renders them inert; of this group the chlorinated antiseptics are the most striking examples. This action of these chlorinated antiseptics is to be attributed chiefly, as pointed out by Dakin, to their affinity for the amino group of the protein molecule.

THE FACTORS CONCERNED IN THE APPEARANCE OF NUCLEATED RED BLOOD CORPUSCLES IN THE PERIPHERAL BLOOD.

II. Influence of Procedures Designed to Increase the Rate of Blood Flow through the Blood-Forming Organs—Hemorrhage and Infusion.*

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In a previous paper the authors (1) have shown that the appearance of nucleated red corpuscles in the peripheral circulation is independent of the increased blood flow produced by exercise or by vasomotor paralysis. A slight increase in the number of these cells may be obtained, but one which, when thoroughly analyzed, never gives the picture of a true normoblast crisis. In the same paper it was said that the experiments on increased blood flow were designed to control more radical perfusion experiments by means of which cellular extrusion from the marrow could be studied directly. The interesting feature of normoblast appearance is its relation to blood regeneration, its reliability as an index of new formation. Perfusion work upon the marrow cannot be carried through a long enough period to give direct evidence of blood regeneration through increased red cell counts. It is therefore necessary to make use of the appearance of nucleated red cells as indicating the operation of stimuli for regeneration, provided this appearance can be shown to be an acute and reliable reaction.

In addition to a further demonstration of the fact that increased blood flow through the bone marrow has in itself no influence upon

* The expense of this research was in part defrayed by a grant from the Bache Fund of the National Academy of Sciences.

normoblast appearance, the present paper is designed to give a thorough picture of the nucleated red cell crisis, its time of appearance, duration, relation to marrow hyperplasia, etc. It would seem that nothing could be added to the literature of secondary anemia brought about by large, repeated hemorrhages. We had hoped on undertaking work upon the problem of blast appearance to find data which would place the reaction with complete accuracy. But such data do not exist so far as we have been able to ascertain. Tables giving the rate of regeneration in relation to hemoglobin formation are numerous, but neither these nor other studies give adequate pictures of the relation of nucleated red cells to regeneration.

Rieder (2) in his study of leukocytosis gives one instance in which normoblasts were found in the blood of a dog 24 hours after hemorrhage. This statement is unaccompanied by control figures of blood composition before blood loss.

Koeppe (3) finds nucleated red cells 23½ and 48 hours after hemorrhage in two rabbits. Here again there is lack of thorough examination before operation.

Zenoni (4) attempts to ascertain whether the appearance of nucleated red cells in the circulation is a result of the decrease of the blood mass through hemorrhage, or whether it is a hematopoietic reaction. Four dogs, two rabbits, and five guinea pigs were used, and care was taken that no normoblasts were present in the circulation before hemorrhage. The guinea pigs and rabbits were bled once from the carotid artery in large amount and films were made from the subcutaneous blood immediately and several hours after the operation. In one guinea pig normoblasts were found after 20 hours, in the two rabbits after 48 hours, and in one dog, treated in the same way, after 18 hours. No saline infusion was given in these cases. The remaining three dogs were bled repeatedly at one operation, the blood being defibrinated and returned. One showed normoblasts in 1½ hours, another in 5½, and the last in 7½ hours. Throughout the entire work there are no tables indicating the number of specimens examined, nor is there any note upon the character of the anesthesia. The hemorrhage and return of defibrinated blood involved a protracted operation. We have found that ether anesthesia, unless exceedingly brief, will cause the appearance of normoblasts with the familiar leukocytosis which is associated with this anesthetic. We may therefore summarize Zenoni's work in the conclusions that simple hemorrhage in rabbits, guinea pigs, and one dog required a considerable interval in order to cause blast appearance, and that rapidly repeated hemorrhages with return of defibrinated blood in dogs caused these cells to appear quickly. We have never repeated these last observations of Zenoni's, since they seemed to us to involve many possibilities aside from those he conjectured. The procedure undoubtedly gives repeated closing down and opening up of the marrow vessels as the circulation is depleted and replenished. This, of course, means periods of decreased and possibly in-

creased blood flow, but unless done under sterile conditions, without defibrination and the consequent introduction of serum and hemoglobin with their possibilities of leukocytosis production and accompanying blast appearance, it seems to us to lack the controls necessary for conclusive deductions.

Lazarus (5) states that nucleated red cells do not appear in human blood until the 2nd or 3rd day after hemorrhage.

Von Willebrand (6) in a series of observations on posthemorrhagic anemia in dogs and rabbits makes the following comment:

> Nucleated red corpuscles were observed after venesection in all the specimens of blood examined, but it must be stated in confirmation of the work of Timofejewsky, and Schauman and Rosenquist, that similar cells were found in three out of nine normal animals examined—dogs and rabbits—prior to venesection, although in these cases the nucleated cells were not numerous, the maximum being 4 in 10 sq. mm.
>
> The cells were usually not present in large numbers after venesection. As a rule they were most numerous during the first few days following the operation, and then disappeared gradually from the blood. I have never been able to observe so numerous an invasion of these cells as von Noorden observed and described under the term "blood crisis."
>
> The exact time of appearance of the nucleated red cells after bleeding cannot be readily determined, inasmuch as these cells may be present normally in the blood. In Experiment 3 relatively numerous cells (21 in place of 2 before venesection) appeared as early as 8½ hours after the operation. All the nucleated cells were of the normoblast type. In size they resembled normal, unnucleated blood corpuscles.

Bunting (7) has compared the blood picture in a rabbit rendered anemic through repeated hemorrhages with that of ricin and saponin poisoning. He found 15 normoblasts per c. mm. 24 hours after the first of two hemorrhages totalling 20 cc. After 26 hours, 10 cc. more blood having been taken on the 24th hour, the number had risen to 108 normoblasts per c. mm. In a second case 12 cells were found 23 hours after a hemorrhage of 45 cc. In neither of these instances were counts recorded at short intervals immediately following the blood loss.

EXPERIMENTAL.

Dogs only were used in the experiments and since, as will be seen, the observations were carried over a long period, particular care was taken to keep the animals in good physical condition. They were fed on a diet of meat, bread, and dog biscuit. Except for an attack of acute and fatal distemper in Dog 1 none of the animals showed illness or suffered loss of weight. In addition to the five dogs whose records form the basis of this paper, we have bled with immediate infusion and bled without infusion six other animals, following the egress of cells at ½ hour intervals for from 8 to 10 hours after the

operation. As the results thus obtained coincide with those obtained with the five animals subjected to more or less continuous observation we do not include them specifically in any of the data presented.

Charts of three of the five dogs of this series are given in this paper (Text-figs. 1, 2, and 3). In the case of the first two, we did not at first realize the need for daily observations so that their records are incomplete, but as far as they go they amply confirm the published data.

It was our original intention to bleed and infuse, studying the blood composition before the operation and at repeated, short intervals after it Experiments with the isolated tibia of the dog showed that hemorrhage and immediate infusion, with consequent blood dilution, will increase the blood flow through the bone often fivefold. Could it be shown that the sudden increase dislocated marrow cells? We have already commented upon the fact (1) that if the marrow vessels of all mammals are as incomplete as those pictured for the rabbit, allowing the normal blood current to wander through a loose network of cells, there should be easy dislocation of such cells under the influence of a markedly increased blood flow. That the dislocation does not occur readily has been indicated by the literature cited, but not proved, because of lack of precise following of the blood changes. It is noticeable that in none of the experiments cited has there been hemorrhage with immediate saline infusion. It is a question how long blood volume restoration by intravenous salt solution lasts. But there is no doubt that for the 1st hour following hemorrhage and infusion the volume of blood passing through the bone marrow is greatly increased. Boycott and Douglas (8) have shown extremely rapid restoration of blood volume in rabbits subjected to large hemorrhages and not infused. Dogs apparently restore blood volume more slowly, though Hünerfauth (9) found no difference between dogs and rabbits. It is possible that this rapidity of restoration indicates the reason our experiments upon blast extrusion show no variation whether or not salt solution was given. There is a frequent small increase in nucleated red cells immediately after hemorrhage, determined apparently by the fact that all intravascular blood cells are in the active blood current at this time and the true blood composition can, therefore, be properly appreciated on examination of specimens from the capillaries.

Boycott and Douglas (8) emphasize the increase in speed of regeneration of hemoglobin which follows repeated hemorrhage and consequent extension of erythrocyte-forming tissue. The effect of hemorrhage in drawing immature red cells into the circulation has not been studied in animals which, because of marked hyperplasia, are known to have a large volume of cell-bearing tissue. The fact that normoblasts accompanying leukocytosis appear with peculiar readiness in children has been commented upon, but the literature is lacking in precise following of the reaction through a series of hemorrhages.

Technique and Immediate Effects of Hemorrhage and Infusion.

Unless otherwise designated blood specimens were taken by subcutaneous puncture after a brief period of exercise. In every case red cell counts, white cell counts, and two films were made, the latter being invariably stained with Wright's stain. Nucleated red corpuscles were enumerated per c.mm. on the basis of the number seen while counting 1,000 leukocytes in the films. All red and white cell counts were made by two of us.

It was found early in the work that the individual hemorrhages were better borne and could be made much more extensive if accompanied by sterile saline infusions. Consequently figures given for the amount of blood removed do not represent whole blood, since in the latter part of the hemorrhage much of the fluid taken out had just been introduced. Under ordinary circumstances after removal of from 200 to 400 cc. of blood an equal amount of salt solution was returned through the same cannula. Then followed another large blood removal and saline injection, and so on until in our judgment the danger limit had been reached. While this method proved most satisfactory for the purpose of protracted experiments when it was essential to run no risk of losing the animal, it obviously leaves the estimate of the size of hemorrhage to the blood counts. In nearly all cases morphine and cocaine anesthesia was used, ether being necessary but a few times.

All operations were done under sterile conditions. Two bleedings could usually be obtained from each external jugular vein, one from each of the carotid arteries, one from each brachial artery, and in

large dogs one from each saphenous artery. This progressive cutting down of the vascular bed never produced permanent ill effects and gave opportunity for ten hemorrhages, more than enough for our purpose. On several occasions we saw temporary circus movements and amblyopia after large hemorrhages, but prompt recovery followed.

Tables I and II illustrate the immediate effect of hemorrhage and infusion upon the nucleated red cell count in a normal dog (Table I), and in the same animal rendered hyperplastic by four hemorrhages occurring at intervals during a period of $2\frac{1}{2}$ months (Table II). On

TABLE I.

Immediate Effect of Hemorrhage and Infusion upon the Nucleated Red Cell Count in a Normal Dog.

Dog 2; female; weight 8.9 kilos; October 21, 1916.

Specimen.	Hour.	Red cells per c.mm.	White cells per c.mm.	Nucleated red cells per c.mm.	Remarks.
	a. m.—p. m.				
1	9.35	4,960,000	12,700	127	1st experimental day.
	9.48				Ether begun.
2	10.09	5,488,000	18,500	203	Specimen 2 taken just before bleeding and after 21 minutes of ether.
Average.....		5,224,000		165	
	10.09–10.23				360 cc. of blood removed in one large hemorrhage; 660 cc. of salt solution returned.
3	10.23	2,776,000	10,300	144	Taken immediately after saline infusion.
4	10.45	3,200,000	8,000	288	Animal rapidly coming out of ether.
5	11.30	3,504,000	11,900	154	
6	12.15	3,816,000	18,200	273	
7	1.00	3,400,000	19,700	157	
8	1.45	3,392,000	20,700	227	
9	2.30	2,992,000	20,200	141	
10	3.15	3,192,000	20,800	166	
11	4.00	3,000,000	17,800	142	
Average.....		3,252,000		188	

October 21, 1916, Dog 2 in two observations showed an average of 165 nucleated red cells per c.mm. After a hemorrhage and an infusion of salt solution which reduced the red cell count from 5,224,000 to 3,252,000, nine observations during a period of $5\frac{1}{2}$ hours immediately following the hemorrhage gave an average of 188 nucleated red cells per c.mm. of blood. This increase, which becomes relatively larger when considered from the point of view of proportion of nucleated red cells to total red cell count, is nevertheless too small, in our opinion, to be regarded as representing a real dislocation of cells from the marrow. We believe it to have been due to a profound stirring up of the circulation following the infusion, and to a consequent more even distribution of cells throughout the peripheral area—a phenomenon such as we have shown to occur with exercise (1). This belief is further strengthened by the figures given in Table II, taken from the same animal.

On January 15, 1917, Dog 2, rendered hyperplastic by four fairly severe hemorrhages, was again bled and infused. Two observations before hemorrhage showed no nucleated red cells. Twelve observations following the infusion and extending over a period of 6 hours gave an average of 5 nucleated red cells per c.mm. Ten of these twelve observations showed no blasts at all. This insignificant increase in an animal whose marrow was presumably richly provided with nucleated red cells, strengthened our belief that the increased rate of blood flow following hemorrhage and infusion is not a procedure which will dislocate cells from marrow.

Table III summarizes briefly the immediate results of numerous hemorrhages in Dogs 3, 4, and 1, giving the number of specimens taken before and after the hemorrhage on each experimental day, the number of hours after hemorrhage during which the observations were made, and the average red cell, white cell, and nucleated red cell count before and after each hemorrhage. These figures confirm those taken from Dog 2. In thirteen out of nineteen cases a slight increase occurred in the number of nucleated red cells following the hemorrhage and infusion; in five cases a slight decrease in the number of blasts followed the hemorrhage; in one instance there was no change.

TABLE II.

Nucleated Red Cell Count in a Dog Rendered Hyperplastic by Four Hemorrhages at Intervals during a Period of 2½ Months.

Dog 2 (continued); January 15, 1917.

Specimen.	Hour.	Red cells per c.mm.	White cells per c.mm.	Nucleated red cells per c.mm.	Remarks.
	a. m.—p. m.				Animal bled and infused on Oct. 21, 1916, Nov. 10, Dec. 14, and Jan. 1, 1917.
	8.50				1.2 cc. of 3 per cent solution of morphine sulfate subcutaneously.
1	8.55	7,528,000	11,500	0	
2	10.20	7,096,000	15,800	0	
Average.....		7,312,000		0	
	10.20–10.40				Bled 630 cc. from external jugular vein; 1,100 cc. of salt solution infused.
3	10.40	2,432,000	17,200	0	
4	11.00	3,120,000	13,000	0	
5	11.30	3,284,000	17,600	0	
6	12.00	2,912,000	19,100	19	
7	12.30	3,176,000	19,400	0	
8	1.00	3,216,000	26,800	0	
9	1.30	3,286,000	24,250	48	
10	2.30	3,028,000	27,600	0	
11	3.00	3,008,000	30,100	0	
12	3.45	3,056,000	25,800	0	
13	4.30	2,944,000	28,000	0	
14	4.45	3,072,000	30,800	0	
Average.....		3,044,000		5	

The True and the Pseudocrisis and the Time of Their Occurrence.

We have just pictured the immediate result of hemorrhage and infusion. On inspection of Text-figs. 1, 2, and 3 it is evident that the slight increase in nucleated red cells occurring immediately after

TABLE III.

Immediate Effect of Numerous Hemorrhages upon the Red Cell, White Cell, and Nucleated Red Cell Count.

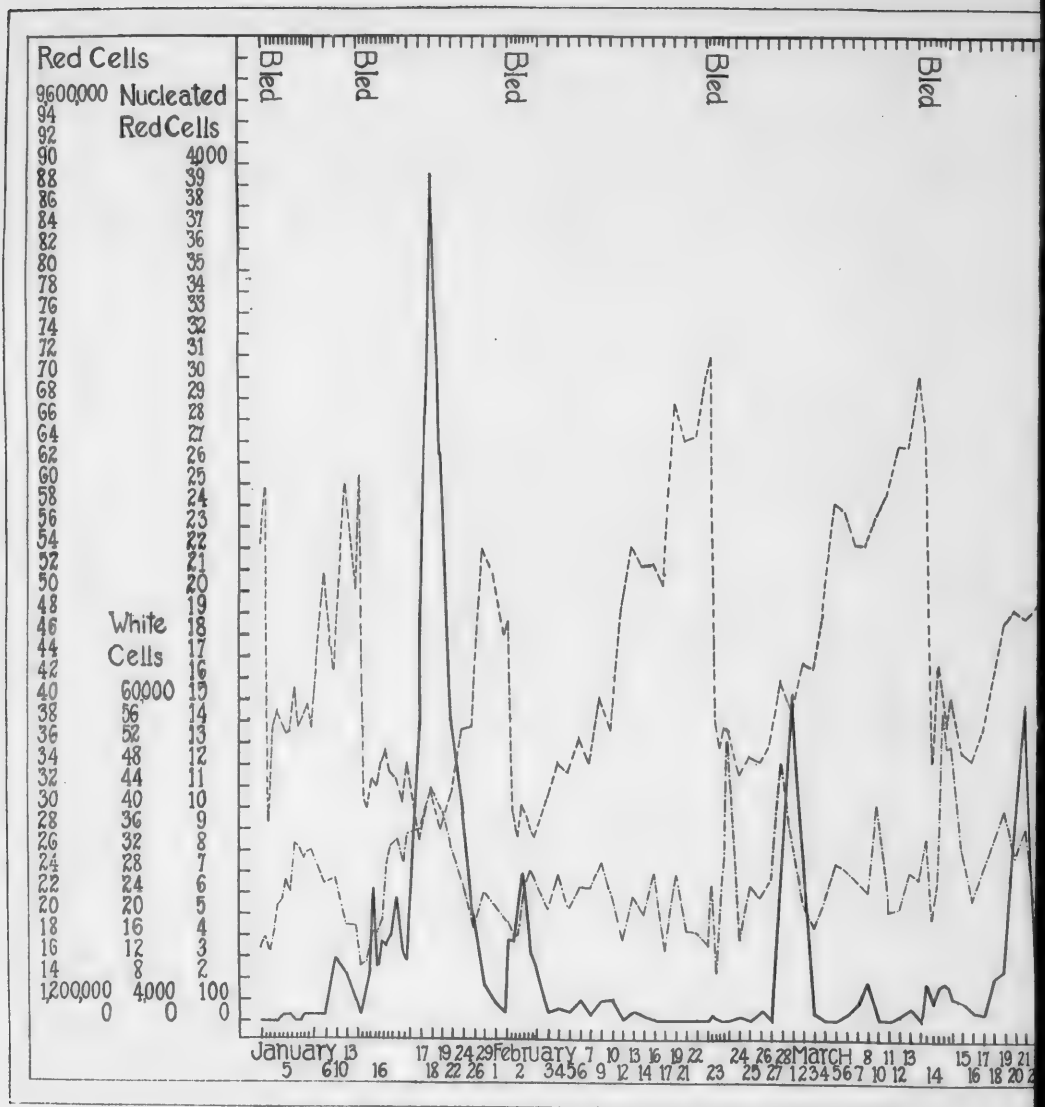
Date.	No. of specimens taken.		Duration of observation after hemorrhage.	Average No. of red cells per c.mm.		Average No. of white cells per c.mm.		Average No. of nucleated red cells per c.mm.	
	Before hemorrhage.	After hemorrhage.		Before hemorrhage.	After hemorrhage.	Before hemorrhage.	After hemorrhage.	Before hemorrhage.	After hemorrhage.
				Dog 3.					
1917			*hrs.*						
Jan. 5	2	11	5¼	5,696,000	3,653,000	14,700	25,000	0	15
" 16	2	10	6	5,556,000	3,261,000	14,000	23,700	59	347
Feb. 2	2	6	5½	4,664,000	2,852,000	18,400	22,300	206	450
" 23	2	4	3½	7,122,000	3,720,000	19,800	29,600	12	2
Mar. 14	2	5	6¼	6,788,000	3,928,000	30,300	42,400	85	133
" 29	1	4	5¼	6,592,000	3,378,000	21,400	36,300	107	102
Apr. 16	2	3	5	7,040,000	4,058,000	23,200	36,300	0	57
May 2	1	3	4	7,416,000	4,834,000	14,400	32,700	14	26
				Dog 4.					
Feb. 9	2	6	5¼	8,812,000	6,384,000	17,000	21,200	0	0
" 20	2	6	5	6,113,000	5,214,000	20,700	38,200	0	266
Mar. 9	1	4	4¼	5,928,000	2,618,000	34,100	28,100	0	47
" 28	2	4	5	6,120,000	3,001,000	14,300	18,300	0	25
Apr. 17	2	3	5	6,772,000	3,320,000	15,100	30,500	14	55
May 4	1	2	2¼	7,040,000	5,463,000	17,800	28,300	0	52
				Dog 1.					
Mar. 7	1	6	5	6,072,000	4,042,000	28,300	18,000	28	2
" 16	1	5	5¼	5,048,000	2,724,000	18,000	19,400	54	65
Apr. 2	2	4	4¾	5,860,000	3,688,000	21,300	28,700	205	89
" 18	2	2	2½	6,158,000	4,080,000	13,600	16,700	423	245
May 7	1	1	½	6,124,000	3,360,000	16,600	8,900	33	320

hemorrhage never reaches a height comparable with that attained later in the curves, just before pronounced red cell increase. This first small addition of normoblasts is well seen in Text-fig. 1, Dog. 3, February 2, 1917. The result of hemorrhage and infusion on this day has been to bring into the active circulation every available cell. The situation is comparable with that seen on immediate exposure to low barometric pressure when polycythemia occurs at once and

expresses the full cell count of the animal at the moment. Later there is a formative polycythemia with the reestablishment of a reserve of red cells. In the same way, with the speeding up of the circulation which occurs immediately following hemorrhage and infusion, every available cell begins to circulate actively. The situation is exactly the one presented by exercise, and we designate the minor increases in nucleated red cells occurring after exercise and immediately after hemorrhage and infusion as pseudocrises. These pseudocrises do not foretell rapid regeneration and it is even doubtful in our minds whether the cells found in them really leave the marrow pulp. They are more probably in the circulation when the hemorrhage occurs.

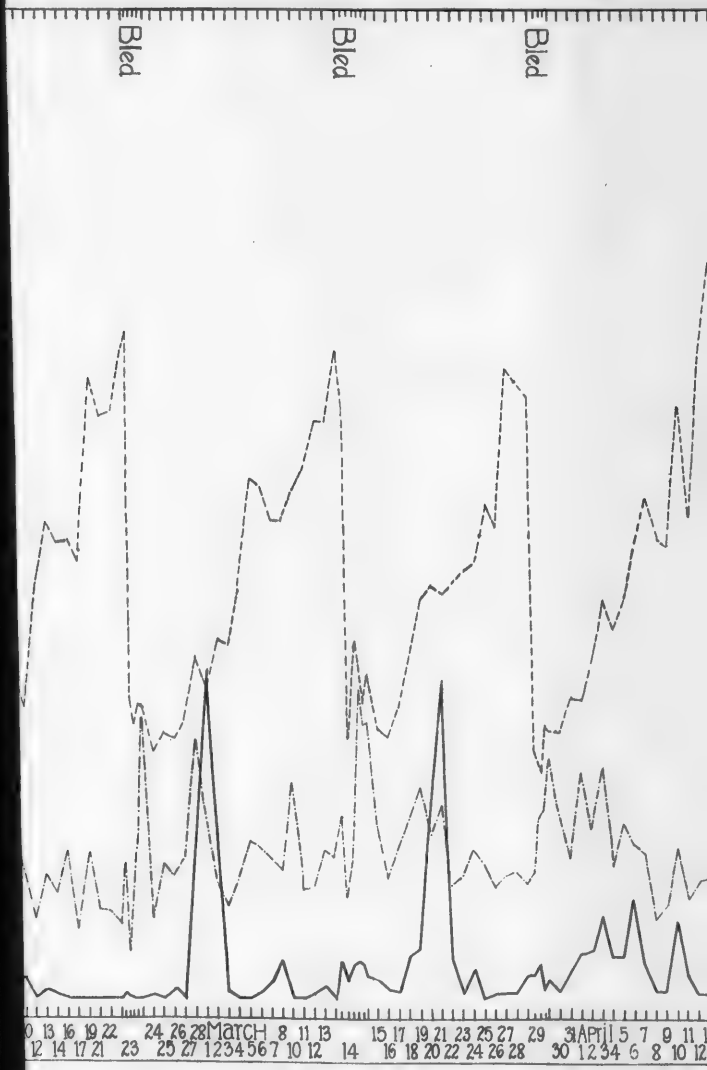
Other examples of the pseudocrisis are seen in Text-fig. 1, Dog 3, March 14 and 29. In these two instances the increases are less than on February 2 and they are preceded by periods of low normoblast content, while that of the earlier date is the result of hemorrhage just at the termination of an extremely large, true formative crisis. Even in the presence of the most advanced hyperplasia, evidenced by autopsy, the immediate effect of blood dilution and increased blood flow through the blood-forming organs is not to dislocate normoblasts but only to give a truer picture of the vascular content of these cells at the time of the operation.

True crises are easily recognized on the charts and their relation to red cell regeneration is obvious. They occur and disappear with great rapidity. This is particularly conspicuous in Text-fig. 3, Dog. 1. The first true crisis here begins on March 21 and ends on the 23rd; the second crisis, beginning April 13, lasts 3 days, in the first two of which the normoblasts increase from 434 to 8,210 per c.mm. It is plain that the crisis is always related to a favorable event, namely active red cell regeneration, but it is equally plain that the true formative crisis is not a necessary accompaniment of regeneration. Indeed, the transiency of the phenomenon, coupled with the fact that as animals are bled repeatedly they lose the tendency to show crises, emphasizes most strongly the fact that every effort is made to avoid this loss of nucleated red cells into the general circulation. A crisis usually occurs after the second hemorrhage. In the three charts presented this is marked. In two cases, Text-figs. 2 and 3, the crisis is largest after the third hemorrhage and then, with regeneration



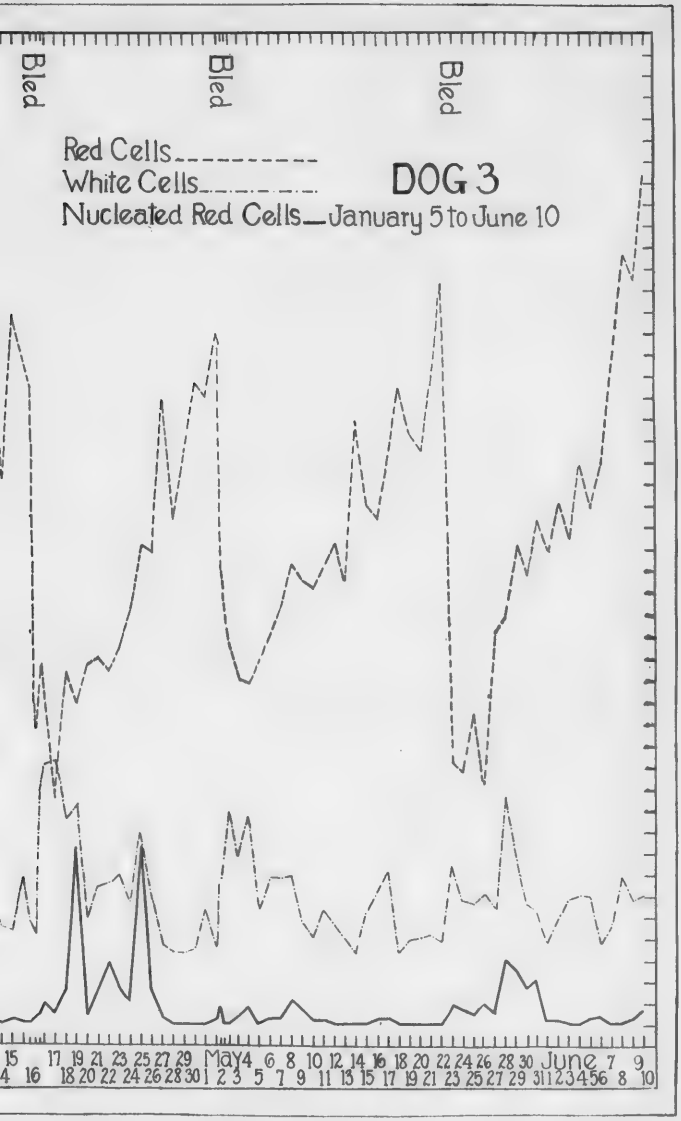
TEXT-FIG. 1. Graphic picture of the daily red cell, white cell, and nucleated red cell counts of Dog 3 during
nates represent cells per c.mm. of blood; the abscissæ represent days of the month. When a number of obse
dicated by short vertical upstrokes on the line of the abscissæ included between two long upstrokes, which
single long upstrokes on the line of the abscissæ.

Contrast the pseudocrises of blasts immediately following hemorrhage and infusion with the true blast cr
regenerate without crises and the steadily increasing overregeneration of red cells as the hemorrhages continue.



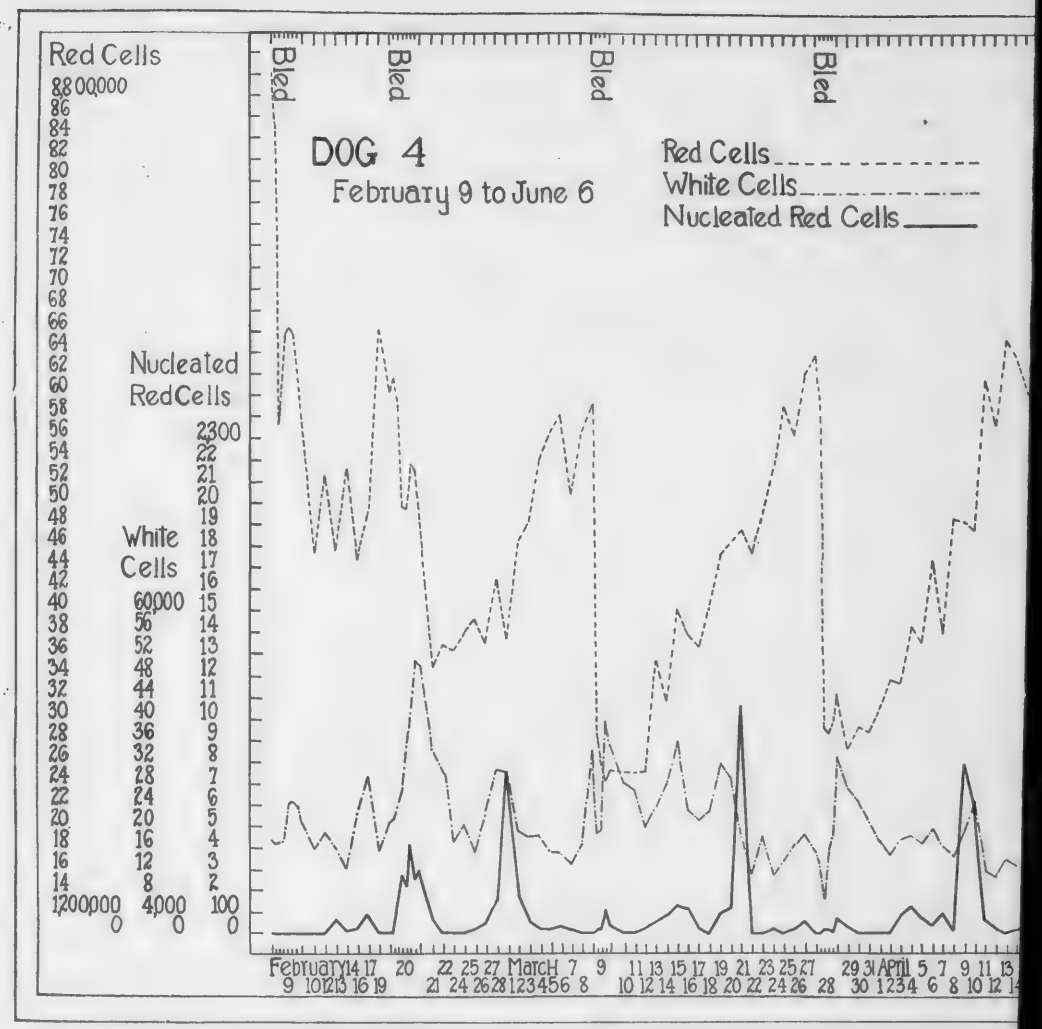
and nucleated red cell counts of Dog 3 during a period of 5 months, showi
days of the month. When a number of observations were made on the
included between two long upstrokes, which indicate the first and the l

emorrhage and infusion with the true blast crises occurring during active
tion of red cells as the hemorrhages continue.

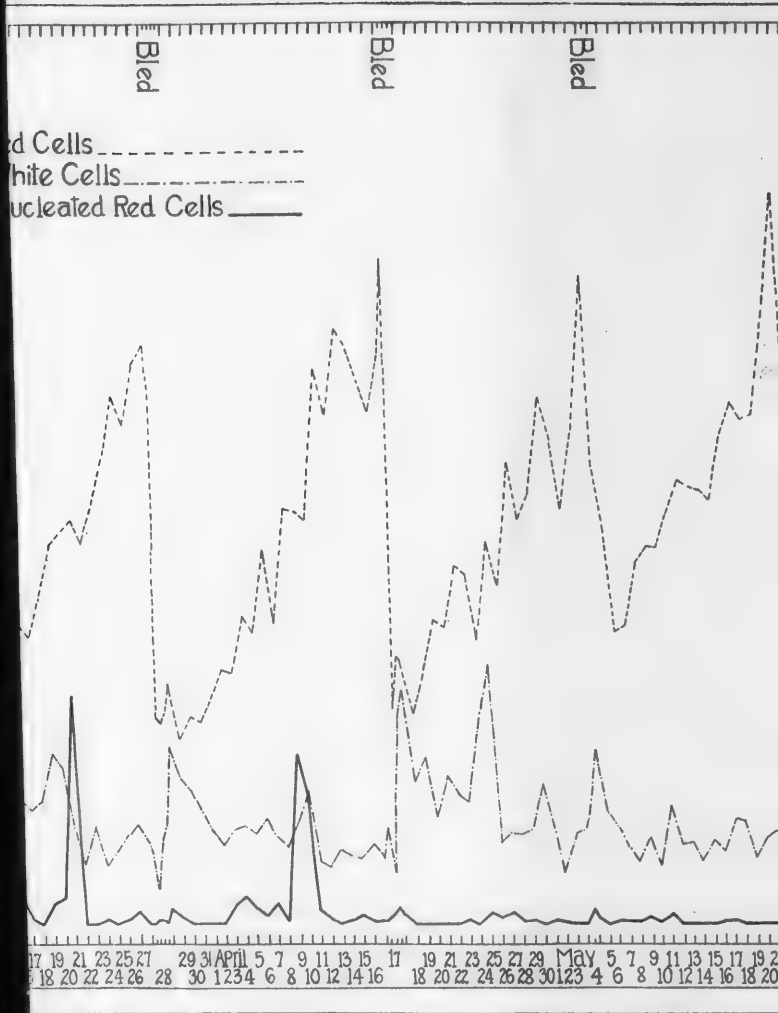


g the immediate and subsequent effects of nine hemorrhages. The ordi-
ame day immediately following a hemorrhage and infusion, they are in-
st observation of the day. Single daily observations are indicated by

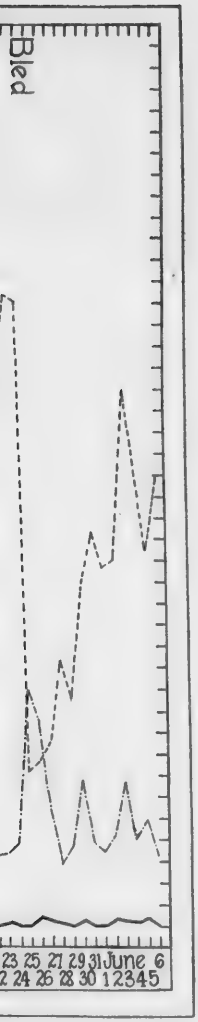
red cell regeneration. Note the gradual development of the ability to



TEXT-FIG. 2. Curve of the daily red cell, white cell, and nucleated red cell counts of Dog 4 during a pe
seven hemorrhages. The same features in the curve are to be noted as in Text-fig. 1. The initial high red



ed cell counts of Dog 4 during a period of 4 months, showing the immediate and
in Text-fig. 1. The initial high red cell count is probably due to a fright polycyt



subsequent effects of
emia.

occurring rhythmically and rapidly after succeeding hemorrhages, there is a progressive decrease in the size of the crises. This gradual acquirement of a power to hold nucleated red cells in the face of rhythmic blood loss is an adaptation which might be expected. While dogs frequently show many nucleated red cells in the peripheral blood and in this case usually display the largest crisis after the first hemorrhage, they invariably begin to regenerate without crises as bleedings are continued. It is unfortunate that in our experiments the hemoglobin content was not followed in relation to this phenomenon, since the failure of hemoglobin regeneration at a rate comparable with cellular regeneration must produce a considerable color anemia in animals treated as these have been. The presence of a low hemoglobin has, however, had no effect on the rate of cell regeneration, and we may mention the fact that Neudörfer (10), one of the first to describe normoblast crises, observed them in cases of chlorosis.

A further question which naturally arises in relation to these sudden floods of nucleated red cells is: What is their fate? They disappear as quickly as they appear. As far as we know, their fate is entirely unexplained. Zuntz and his associates (11) believe that they are destroyed if present freely in the blood stream, but do not explain how. Our own observations, in which we have seen them resist the pounding of a mechanical glass perfusion pump through many hours without reduction in number, lead us to believe that they are as resistant to trauma as are non-nucleated cells.

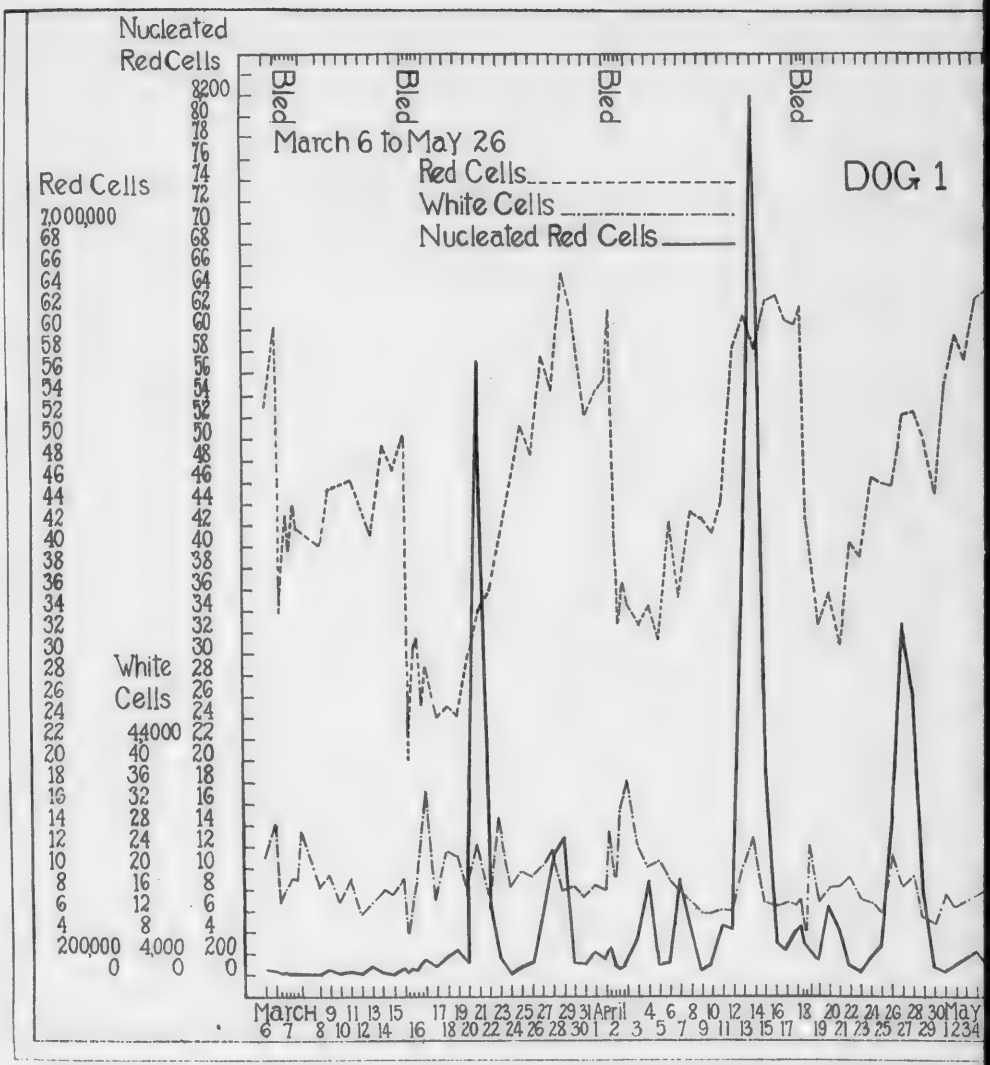
Concomitant Behavior of Red and of White Cells.

Those who have watched blood regeneration closely have been impressed with a tendency towards simultaneous movement of red and of white cells from the marrow. A high, sudden leukocytosis, polymorphonuclear in character, is frequently accompanied by a few nucleated red cells. Hough and Waddell (12) in studies of regeneration from single large hemorrhages in dogs state:

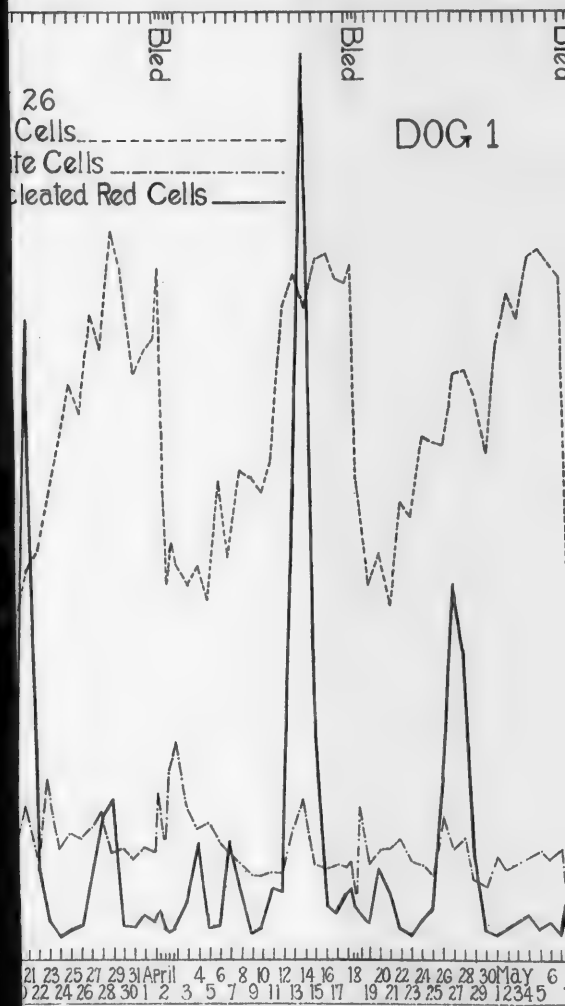
"Each rise in the erythrocyte count is accompanied by a distinct rise in the leucocyte count either on the same or the preceding day. It is believed that, in general, when other causes of leucocytosis are controlled or absent, the leucocyte count may be taken as an indication of the degree of activity of the blood forming organs."

They do not report upon stained films, however, and have evidently observed increases in leukocyte count such as frequently accompany or just precede blast crises in our experiments. In the case of Dog 3, Text-fig. 1, the curves of nucleated red cells and leukocytes follow one another closely. Dog 4, Text-fig. 2, also shows increases in leukocyte count just before or with the blast crises of March 1 and 21 and April 9. However, during the period from April 12 to June 6, in which three large hemorrhages are given and recovered from, there are no normoblast crises, and with one exception no marked leukocytoses except those immediately after the hemorrhages. On April 24, 25, and 26 a definite leukocytosis occurs, caused by a localized abscess in the neck wound made in the bleeding on April 17. The white cells in this case have appeared in increased numbers and independently of normoblasts. Dog 1, Text-fig. 3, shows insignificant leukocytic increases with the blast crises, but nothing which alone would be significant as pointing to a regenerative tendency except possibly the leukocytosis beginning on April 13 and ending on the 15th. We may therefore agree with Hough and Waddell in feeling that a sudden and unaccountable leukocytosis may foretell rapid regeneration, but the reaction by no means always occurs before regeneration. Indeed, a tendency to avoid leukocytosis in the typical crisis position in the blood regeneration curve occurs as marrow hyperplasia advances. That the animals are well supplied with leukocytes is indicated by the vigorous posthemorrhagic leukocytoses which are seen to follow the hemorrhages throughout the entire course of the observations, and also from the readiness of development of the infectious leukocytosis in Dog 4.

Lastly, we should mention briefly posthemorrhagic leukocytosis. We have been impressed by the reliability and the extreme rapidity with which this reaction comes on. We have frequently seen the white count double in 2 hours. This is not a change in distribution of the white cells, since we have found the increase in specimens taken at the same time from heart, vein, and capillary blood. It is a real increase in the number of circulating white cells, and emphasizes the fact that the marrow is a reservoir of leukocytes in contrast to its relation to the red cells. Adult red cells are extruded minute by minute as fast as they are formed, and there is every tendency to



TEXT-FIG. 3. Curve of the daily red cell, white cell, and nucleated red cell counts of Dog 1 during the immediate and subsequent effects of six hemorrhages. Owing to the extremely large true blast crises the scale used in indicating the number of normoblasts is one-half the scale used for Dogs 3 and 4. T are the same as those in Text-figs. 1 and 2.



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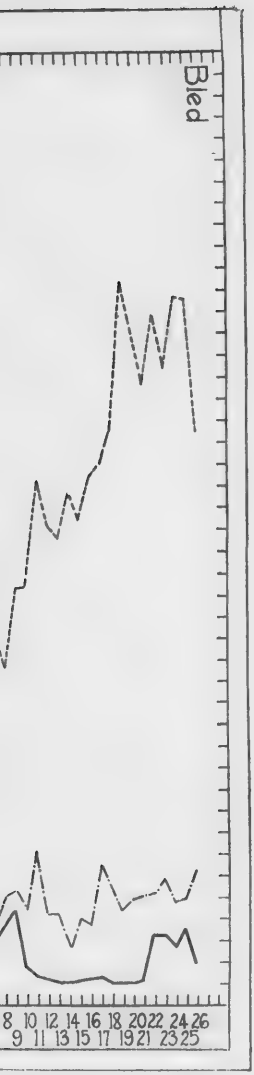

eriod of 3 months, showing
ich occurred in this animal
chief features in this curve

resist undue haste in extrusion, as shown by the increasing power to hold nucleated cells which develops after a certain number of hemorrhages. But the marrow has an emergency function in relation to the leukocytes, a capacity to discharge the cells independently and rapidly, at times when many normoblasts are present, and under the influence of a procedure—hemorrhage—which brings about gradual increase in red cell counts.

Numerical Overregeneration of Red Cells and Influence of Repeated Hemorrhages on Rate of Regeneration.

Boycott and Douglas (8) have shown that after repeated bleedings in rabbits the rate of regeneration of hemoglobin becomes much greater than after a single hemorrhage, and that overregeneration occurs after single and multiple hemorrhages. The phenomenon of overregeneration of cells appears in our experiments. The high points in the curves become higher and higher as one passes to the right across the charts. This is particularly well seen in Dog 3, but in our opinion is equally true of Dog 4. The initial high red cell count in the latter animal was probably a fright polycythemia such as has been described by Lamson (13). The final regeneration after the bleeding was interrupted on June 6 by the utilization of this dog in a marrow perfusion experiment. Were it not for the fact that Boycott and Douglas have shown that there is a needless overregeneration of hemoglobin after hemorrhage, one might think that the polycythemic tendency which the repeatedly bled animals have shown was certainly due to the lag in hemoglobin formation, a multiplicity of cells carrying small amounts of hemoglobin taking the place of fewer cells better supplied. We followed this posthemorrhagic polycythemia for 2 months after the last bleeding in one animal, No. 2, upon whom, after six hemorrhages, all bleedings were discontinued. The red cell count at the end of this time was 9,336,000 cells per c.mm. This persistent polycythemia after repeated hemorrhages is comparable with the slow disappearance of the polycythemia of low barometric pressure. Apparently marrow hyperplasia once established resists disestablishment to a marked degree.

In considering the tendency to regenerate without crises, an adaptation of great interest, we have not met the criticism that such a process

may be due to marrow exhaustion. In all three animals whose records are given, autopsy with subsequent histological examination of the marrow disclosed marked hyperplasia, extension of erythrocyte-bearing marrow into the shafts of the long bones, and many islands of erythroblastic activity. Further, if one takes 6,000,000 cells per c.mm. as the normal red cell count of these animals, it is seen that regeneration to this level takes place in almost the same length of time throughout the entire series of bleedings. It is noteworthy that there is no significant increase in speed of regeneration as marrow hyperplasia advances, but that at the same time there is no lag in red cell formation. It has not been possible to confirm the work of Boycott and Douglas (8) on increased speed of hemoglobin regeneration by an analogous increase in speed of red cell regeneration. We have shown that after successive hemorrhages regeneration is not delayed, that the process apparently becomes more orderly as evidenced by the failure to find normoblasts in the peripheral blood, and ultimately reaches an abnormal height. Boycott (14) notes the fact that young rats and rabbits with hyperplastic marrow do not extrude nucleated red cells after hemorrhage as do adults. Our experiments with dogs do not entirely verify this since one of our animals, No. 1, a pup weighing 5.2 kilos when first obtained and 8.6 kilos at death $2\frac{1}{2}$ months later, showed large crises of normoblasts when first bled and passed into the state of regenerating without crises in much the same manner as did the adult dogs.

CONCLUSIONS.

1. Hemorrhage with immediate saline infusion causes the appearance in the peripheral blood of a slightly increased number of normoblasts provided normoblasts are already present in the blood stream. Marrow hyperplasia does not intensify this reaction and the cells found probably do not leave the marrow pulp but are in the blood stream at the time of the experiment.

2. The slight increase in cells occurring immediately after hemorrhage and infusion is designated a pseudocrisis. True crises are much more extensive; they tend to occur just before rapid increase in the erythrocyte count and usually towards the end of the 1st week following hemorrhage.

3. Red cells and white cells tend to move from the marrow together, but this association is not invariable.

4. After repeated hemorrhages regeneration occurs independently of the appearance of nucleated red cells in the peripheral blood.

5. Repeated hemorrhages associated with extension of erythrocyte-producing marrow lead to polycythemia but not to a conspicuous increase in speed of regeneration.

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THE MORPHOLOGICAL CHANGES IN THE TISSUES OF THE RABBIT AS A RESULT OF REDUCED OXIDATION.

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The experiments which are described in this paper were undertaken with the idea of determining the morphological changes which might be found in the tissues of animals subjected to the change of a single factor in their chemical environment. For this purpose two series of experiments have been carried out. In one, a decrease in oxygen of the respired air has been the sole factor of change. In the other, the change has been an increase in the carbon dioxide of the air with other factors kept as a constant.

In considering the results of the two series it would seem reasonable to assume that we are dealing with results, direct or indirect, in one case of a reduced oxygen supply to the cells of the body, and in the other of an increased carbon dioxide content of the fluids and tissues of the body. The assumption, however, that the morphological changes found could be produced in no other way is not made. Death of a cell may, for example, be produced by anemia, or by bacterial toxins. A finer analysis might, however, show that in both instances a reduction of vital oxidation was the cause of the lesion; on the one hand through oxygen starvation, on the other through failure to utilize oxygen by interference with the cellular oxidases by the toxin. It is our opinion, then, that oxygen deficiency should at least receive consideration as a cause, in interpreting lesions similar to those we shall describe in the animals subjected to low oxygen tension.

EXPERIMENTAL.

In carrying out the experiments we have used the respiration chamber described by Kolls and Loevenhart.[1]

[1] Kolls, A. C., and Loevenhart, A. S., *Am. J. Physiol.*, 1915–16, xxxix, 67.

In the first set of experiments ten rabbits varying from young to full grown were used (Table I). The duration of the experiments, in which the animals were exposed to low oxygen tensions, was 7, 10, and 4 days respectively. The animals were removed from the cham-

TABLE I.

Oxygen Rabbits.

Animal No.	Weight.			O_2		CO_2	
	Before experiment.	After experiment.	Loss.	Minimum.	Average.	Maximum.	Average.
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*
1	2,100	1,820	280	4.78	7.74	0.80	0.25
4	950	820	130	4.78	7.74	0.80	0.25
9	1,900	1,380	520	3.58	7.21	0.30	0.23
10	940	720	220	3.58	7.21	0.30	0.23
11	1,320	1,000	320	3.58	7.21	0.30	0.23
12	2,080	1,700	380	3.58	7.21	0.30	0.23
13	1,300	987	313	5.47	7.33	0.20	0.20
14	1,660	1,419	241	5.47	7.33	0.20	0.20
15	760	Died.		5.47	7.98	0.20	0.20
16	1,680	"		5.47	7.98	0.20	0.20

Animal No.	Length of exposure to different O_2 tensions.*					Duration of experiment.
	12–10 % O_2.	10–8% O_2.	8–6% O_2.	Under 6% O_2.	Total.	
	hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*1915*
1	19.0	37.5	64.5	35.0	156.0	Oct. 18–25
4	19.0	37.5	64.5	35.0	156.0	" 18–25
9	11.50	86.25	73.0	60.50	231.25	Nov. 8–18
10	11.50	86.25	73.0	60.50	231.25	" 8–18
11	11.50	86.25	73.0	60.50	231.25	" 8–18
12	11.50	86.25	73.0	60.50	231.25	" 8–18
13		18.00	70.5	8.00	96.50	" 23–26
14		18.00	70.5	8.00	96.50	" 23–26
15		1.00	9.00	2.00	12.00	" 23
16		1.00	8.50	2.00	11.50	" 23

* Only O_2 tensions under 12 per cent are considered.

ber not more than once during the progress of the experiment and then only during the time the chamber was being cleaned (about an hour). The number of hours during which the rabbits were subjected to different tensions of oxygen and carbon dioxide together with the

average tensions of the gases is recorded in Tables I and II. The carbon dioxide determinations were made with Haldane's portable apparatus for the analysis of mine gases. The oxygen determinations were made with a Hempel phosphorus pipette. At the termination of the experiments the animals were killed and immediately autopsied. The tissues were preserved in formalin, Zenker's solution, and 95 per cent alcohol. All the rabbits were weighed before they were put into the chamber and immediately before they were killed. A portion of the record of an experiment is given here.

Experiment 1. Reduced Oxygen.—Nov. 8, 1915.

Rabbit 9, white, male; weight 1,900 gm.

Rabbit 10, black, female; weight 940 gm.

Rabbit 11, speckled, male; weight 1,320 gm.

Rabbit 12, brown, male; weight 2,080 gm.

2.55 p.m. Experiment started. Burned hydrogen flame in the box to reduce oxygen rapidly, and started pump. All the animals were eating and seemed normal.

3.25 p.m. Rabbits becoming drowsy and slightly dyspneic.

3.30 p.m. $O_2 = 9.61$ per cent.

3.37 p.m. H turned off and O_2 turned on.

3.45 p.m. $O_2 = 9.06$ per cent. Animals becoming more dyspneic and drowsy. No. 10 seems to be most affected. Nos. 11 and 12 eating again.

4.15 p.m. $O_2 = 9.11$ per cent. Readjusted control for O_2 intake. Turned O_2 cup down slightly.

5.00 p.m. $CO_2 = 0.25$ per cent.

5.15 p.m. $O_2 = 9.01$ per cent.

7.15 p.m. $O_2 = 8.47$ per cent. Animals seem to be sleeping.

8.05 p.m. $O_2 = 8.26$ per cent. Readjusted O_2 intake.

10.40 p.m. $O_2 = 8.65$ per cent.

Nov. 9, 8.30 a.m. $O_2 = 9.28$ per cent. Readjusted O_2 intake. $CO_2 = 0.30$ per cent. Animals seem to be in good condition. Changed belt on motor.

8.50 a.m. Relative humidity 58 per cent.

9.00 a.m. O_2 off. The animals have been eating.

9.30 a.m. $O_2 = 8.3$ per cent.

11.50 a.m. $O_2 = 6.16$ per cent. Animals becoming very dyspneic.

1.05 p.m. O_2 on.

4.00 p.m. $O_2 = 5.46$ per cent. Readjusted O_2 intake.

4.05 p.m. $CO_2 = 0.20$ per cent.

4.15 p.m. Relative humidity 48 per cent. Drew off sulfuric acid.

7.00 p.m. $O_2 = 7.12$ per cent.

7.30 p.m. $CO_2 = 0.25$ per cent.

8.45 p.m. $O_2 = 7.93$ per cent.

The general data of the experiments are given in Tables I and II.

The symptoms manifested by the animals under different low oxygen tensions were constant in each series of experiments. When the oxygen was reduced to between 10 and 12 per cent, the rabbits became drowsy and slightly dyspneic. As the oxygen tension was reduced further, the dyspnea became more pronounced and was often accompanied by a swaying of the animal from side to side. When the tension was reduced below 7 per cent, the animals refused to eat or drink. Below 6 per cent oxygen, peculiar chewing movements were noticed. The movements of the rabbits became uncertain and it was difficult for them to retain the normal sitting posture. Inability to use the hind legs was noted in nearly all the rabbits. A characteristic symptom was the position of the head, which was thrown far back, possibly in an effort to make the act of breathing easier. One rabbit became paralyzed and appeared about to develop convulsions, when it was removed from the chamber. It recovered completely after a few minutes in normal atmosphere, but when put back into the chamber, the symptoms returned and the animal was discarded.

It is evident that three factors must be taken into account in interpreting the lesions: the low oxygen tension, the increased cardiorespiratory activity, and the lessened intake of food. The consistent loss of weight by all the animals indicates clearly that although the animals are not active in the cage, the intake of food is not sufficient for their vital demands. The general behavior of the animals indicates further that the compensatory effects, by the cardiorespiratory and hematopoietic systems, are not adequate to meet the severe demand made by the low percentage of oxygen which was maintained throughout the series of experiments.

The lesions of the animals at postmortem examination show the slight degree of change found in the various tissues. While the animals vary in the susceptibility of the different organs to oxygen deficiency there is a striking uniformity in the general picture. A single postmortem protocol may therefore suffice.

Rabbit 1.—Male; weight 1,820 gm. Killed Oct. 25, 1915.

Subcutaneous, mesenteric, and retroperitoneal tissues practically free from fat. Serous cavities free from excess of fluid.

Heart.—Large and pale. Left ventricle 5 mm. thick; right ventricle 1.5 mm. Muscle is uniformily pale on section.

Lungs.—Left: Pale and air-containing throughout and exudes a small amount of frothy serum from the lower lobe upon pressure. There are several punctate hemorrhages in the lower lobe. The border of the lung is slightly emphysematous. Right: Similar to left and also shows two small hemorrhages.

Spleen.—Measures 57 by 13 by 5 mm.; is of a deep purplish color and has a pitted and wrinkled surface. On section, the Malpighian corpuscles are of normal size. The pulp is increased in amount and is quite firm.

Liver.—Weight 59 gm. The capsular and cut surface are uniformly and finely mottled, due apparently to a dark appearance of the center and a light opaque appearance of the periphery of the lobule. The gall bladder is filled with dark green bile.

Kidneys.—Left: Measures 35 by 21 by 15 mm. Capsule is not adherent. The surface of the kidney has a uniform grayish pink color, which also characterizes the cortex on section. The medulla is paler. The cortex measures 4 mm. in thickness and has a regular striation. Right: Measures 34 by 22 by 12 mm. and resembles the left in every detail.

Adrenals.—Left: Measures 9 by 7 by 2 mm. On section, cortex measures 1 mm. in thickness. Medulla is linear and about 0.5 mm. in width. Right: Measures 10 by 7 by 2 mm. and is similar to left on section.

Pancreas.—Appears normal.

Aorta.—Intima is slightly opaque and appears thickened.

Thyroid.—Small and pale. External parathyroids small.

Bone Marrow.—From center of femur is of a deep opaque grayish red color, fairly firm, and gives no gross evidence of adipose tissue.

Brain and Cord.—No abnormality evident.

The tissues were fixed in Zenker's fluid, in 10 per cent formalin, and in alcohol.

Microscopic Examination.—

Heart.—Blood and lymph vessels throughout the section are distended and there is some edema of the tissues. The muscle fibers throughout are swollen, granular, and vacuolated, but the lesion becomes extreme only in the inner third of the heart wall. Here the fibers on cross-section appear to have but a single peripheral row of sarcostyles about a vacuole which contains a granular precipitate and in some cases is crossed by delicate fibrils. On longitudinal section the fibers are extremely thin but show the cross-striation. Many of the fibers show hyaline masses which extend usually across the width of the fiber but in some cases appear spherical and of a diameter less than that of the fiber itself. These hyaline masses occur both at the ends of the cells and at intervening points. The nuclei of the fibers stain sharply but appear somewhat shrunken. No fat is shown by osmic acid staining.

Lung.—The vessels are congested. The subpleural air spaces are excessively dilated and their walls appear thin. There are scattered minute alveolar hemorrhages, of antemortem occurrence as shown by the presence of occasional phagocytes containing red blood cells. There is slight edema. The lymphoid tissue is hyperplastic.

Spleen.—There is some hyperplasia of Malpighian corpuscles and of pulp cells as shown by mitotic figures. Both the pulp and its sinuses are distended with cells, chiefly red blood cells, although numerous polymorphonuclear leukocytes are found in the pulp. There are scattered nucleated red cells in the pulp, and in the sinuses are numerous groups of nucleated red cells of all sizes from megaloblast to normoblast, resembling the erythrogenic groups of the marrow.

Liver.—There is a general congestion of the vessels. The liver cells are most normal in the peripheral zone and next to the portal spaces. Here the cells are swollen and granular, with a tendency to a separation of the granules by ill defined clear spaces. Practically every cell has two nuclei. As one approaches the central vein, there is a tendency for the swollen cell to appear paler, to show a greater clear area crossed by an irregular protoplasmic reticulum, with scattered granules. In some parts of the liver clear-cut small fat vacuoles are found in the cells of the middle zone. In most of the sections the central zone shows necrosis of the liver cells and invasion by polymorphonuclear leukocytes. These areas of necrosis extend from central vein to central vein, about the periphery of the secretory lobule.

Kidney.—The vessels are congested. The cells of the cortical tubules are, in general, swollen until the tubular lumen is practically obliterated. The cells are granular but paler than normally. The granules appear to be separated from one another by serum. The lesion varies in the different cells from this condition to one in which the entire cell appears to consist of a nucleus and a vacuole surrounded by a cell membrane. In some cells the vacuole shows an irregular reticulum of protein material. The lesion appears most marked in the subcapsular region. Some of the tubules show a granular precipitate in the lumen and here and there a hyaline droplet is seen. There is no definite cast formation.

Bone Marrow.—The blood vessels are widely dilated and engorged with red cells. The marrow spaces are practically free from adipose tissue cells. The spaces are occupied by regular groups of nucleated red blood cells and by groups of myelocytes and leukocytes. The erythrocyte-producing centers predominate.

Aorta.—The muscle cells of the media are swollen and show a poorly defined vacuole about the nucleus.

Thyroid.—The alveoli of the thyroid are relatively small. The larger are filled with colloid, but there are many smaller alveoli devoid of colloid and practically without lumen.

Testicle.—There are few mature spermatozoa in the lumina. The germ cells appear to be vacuolated and in some places have pyknotic nuclei.

To summarize, the lesions found in the rabbit are: marked hyperplasia of the bone marrow; extramedullary (splenic) production of red cells; degenerative lesions of parenchymatous cells of the nature of a diminution of cell protoplasm with increase of serum (hydropic or vacuolar degeneration or serous imbibition); necrosis of parenchymat-

ous elements in some organs, especially the liver; hyaline degeneration and necrosis; congestion and edema of organs.

Rabbit 4, cage-mate of the animal described, showed practically the same lesions with two exceptions. The liver was less affected and showed no central necrosis, but instead well marked serous imbibition involving the whole lobule with the exception of an occasional cell close to the portal space. The kidney, on the other hand, gave evidence of a previous injury, in the presence of connective tissue scars, and appeared to be more injured by the oxygen deficiency. The serous imbibition was more marked, and some disintegration of cells and necrosis existed.

Rabbits 2 and 3 of this 7 day period were discarded as they were found to be pregnant when examined post mortem, although their lesions agreed with those described.

In studying the tissues of the rabbits of the 4 day and 10 day periods, similar lesions were found throughout, with but slight individual variations. In the hearts of animals of the 4 day period fatty infiltration was a prominent feature, and in fact was more marked than the vacuolar change. In the 10 day animals visible fat was lacking and the serous imbibition was the prominent lesion. In none of the four animals, however, was it as marked as in those of the 7 day period. This was interpreted as indicating a more complete though still insufficient compensation on the part of the blood and respiratory systems.

A tendency to emphysema of the borders of the lungs was noted in practically all the animals, though it was very slight in Rabbit 11. Minute hemorrhages and slight edema were constant. Hyperplasia of the lymphoid tissue in the lungs was also constant. In addition, in all the animals that were in the cages over 24 hours a mononuclear cell invasion of the lung framework of varying degree was present.

The livers uniformly showed hydropic degeneration of the cells of the mid-zone and central zone. In addition, Rabbits 12 and 14 showed a well marked central necrosis, while Rabbit 11 showed a fatty degeneration in the mid-zone and a serous imbibition in the central zone.

The kidneys of the series uniformly showed parenchymatous and hydropic degeneration. Hydropic degeneration was, however, very slight in Rabbit 14, which showed a well marked fatty degeneration.

The spleens showed, in general, hyperplasia of the Malpighian corpuscles and congestion of the pulp. Groups of erythroblasts were noted in the pulp sinuses in Rabbits 9 and 11. Rabbit 13 showed an excessive amount of hemosiderin in the pulp cells, a condition not found in the others of the series.

The aortæ of the animals generally showed a serous vacuolation of the smooth muscle fibers.

The bone marrow showed a marked hyperplasia in all the animals of the 7 and 10 day periods and less marked in those of the 4 day period. The erythroblastic centers were especially hyperplastic, although there was an increase also in the leukogenetic centers.

There was also a general hyperplastic condition of the thyroid gland in all the animals, with some variation in degree.

The lesions that we found then, in animals subjected to low oxygen atmospheres, may be summarized briefly as: (1) Hyperplastic changes in the bone marrow and in the thyroid. (2) Degenerative changes, with ultimate necrosis, in the elements of the heart and glandular organs. These changes are parenchymatous (albuminous), fatty, and serous (hydropic) degeneration, with some hyaline degeneration, and are most marked in the organs at points which are at the greatest distance from the blood supply; *i.e.*, the central zone of the liver, the inner part of the heart wall.

Before discussing the interpretation of the changes, it seems best to describe the experiments with animals kept at normal oxygen pressure and increased carbon dioxide. In this part of the work we have used only four rabbits, as the results were highly uniform. We find that there are certain similarities and differences as compared with the changes in animals subjected to diminished oxygen supply. Identical precautions were observed in this experiment with those in the series of reduced oxidation experiments in that normal atmospheric pressure, humidity, and temperature were maintained. The number of hours during which the animals were subjected to different carbon dioxide tensions is given in Table II.

The symptoms manifested by the animals in the atmosphere of high carbon dioxide were not so severe as those shown by the animals in the low oxygen atmospheres. The dyspnea was less marked, but the respirations had a peculiar short and jerky character. Through-

out the period, the animals ate and drank normally and showed no loss of muscular power, a condition contrasted to the first group described.

In examining these animals post mortem so little deviation from the normal was found, grossly at least, that it seems unnecessary to transcribe the protocols in detail. In fact, it would seem to suffice

TABLE II.

Carbon Dioxide Rabbits.

Animal No.	Weight.			O_2		CO_2	
	Before experiment.	After experiment.	Loss.	Minimum.	Average.	Maximum.	Average.
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*
5	2,250	1,860	390	15.50	22.38	19.64	7.44
6	1,700	1,500	200	15.50	22.38	19.64	7.44
7	1,360	1,240	120	15.50	22.38	19.64	7.44
8	2,250	2,020	230	15.50	22.38	19.64	7.44

Animal No.	Length of exposure to different CO_2 tensions.										
	Under 1%	1-4%	4-6%	6-8%	8-10%	10-12%	12-14%	14-16%	16-18%	18-20%	Total.
	hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
5	4.0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5
6	4.0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5
7	4.0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5
8	4.0	46.5	25.0	25.0	8.0	10.0	2.0	27.5	5.5	7.0	160.5

The duration of experiment on each of the rabbits was from Oct. 28 to Nov. 4, 1915.

to point out how they differ from those of the reduced oxygen series.

Like the latter, the carbon dioxide animals lost weight and were found to have a minimum of body fat at the time of the postmortem. This occurred even though during the course of the experiment they appeared to eat normally.

The hearts showed no abnormality but the blood in the chambers was dark.

The lungs of the animals showed less emphysema of the free borders

than in the oxygen rabbits. Slight emphysema was noted in Nos. 7 and 8, none in Nos. 5 and 6.

The spleens of the animals showed a reduced amount of pulp which was also pale in contrast to the congested dark red pulp of the animals of the oxygen series.

The livers were of uniform color upon section and showed none of the fine mottling characteristic of the animals of the previous series, with the exception of Rabbit 7, which showed lobules with dark center and light periphery.

The kidneys were similar in appearance to those of the oxygen rabbits. Rabbit 5 showed many depressed cortical scars.

The most striking difference in postmortem findings was in the bone marrow taken from the center of the femur. The hyperplastic marrow of the reduced oxygen series was lacking. The marrow instead was soft, pale, and fatty. The absence of hyperplasia was fully confirmed by microscopic study. The bone marrows showed the presence of many adipose tissue cells and but a few peripheral groups of blood-producing elements.

The microscopic examination of the tissues of this series of animals showed a striking uniformity of lesions which were very slight in all cases and much less pronounced than those in the animals of the reduced oxygen experiments. The lesions were chiefly of a degenerative character and of the nature of the hydropic change noted in the other series.

The hearts uniformly showed, on section, muscle fibers which may be described as somewhat swollen, granular, and vacuolated (serous vacuolation). The vacuolation was never so extreme as in the earlier series. It showed, furthermore, a different distribution, being as well marked in the subpericardial layers of muscle fibers as in the deeper layers. Fatty infiltration was noted also in the hearts of Rabbits 5 and 8.

The lungs showed well marked congestion but no edema, very slight emphysema, and slight lymphoid hyperplasia.

The livers of the rabbits showed swollen, granular cells with a tendency to serous vacuolation. This was well marked in but one rabbit, No. 8, and in it was not of the degree seen in the oxygen deficiency rabbits. Moreover, in the four rabbits of the carbon dioxide series, the

change was more marked in the peripheral and mid-zone than in the central zone, the point of most marked change in the oxygen series.

The kidneys of the four rabbits showed a moderate swelling of the parenchymatous elements with increased granulation and some vacuolation. The last feature was well marked in Rabbits 6 and 8. The kidneys of Rabbits 5 and 8 showed old cortical scars and hyaline casts in the collecting tubules. Rabbit 7 also showed casts, and an albuminous precipitate in the glomerular capsular space.

The spleens showed well marked hyperplasia of the Malpighian corpuscles, and of pulp elements, with but moderate congestion. The spleen of Rabbit 5 showed an excess of hemosiderin in the pulp.

The thyroids showed a slight hyperplasia.

The aortæ showed slight fatty degeneration of the muscle fibers by a Sudan III stain, and serous imbibition was noted in the aorta of Rabbit 7.

The adrenals appeared normal.

The characteristic lesions in the rabbits exposed to a high carbon dioxide atmosphere may be summarized as very slight and of a degenerative character, affecting the parenchymatous cells of the heart and important glandular organs. This lesion which is of the nature of a serous imbibition, or vacuolar degeneration, is much less marked than in the animals exposed to an atmosphere deficient in oxygen and fails to show the same distribution, particularly in the heart and liver. Evidence of hyperplasia of the bone marrow is entirely lacking.

DISCUSSION.

In considering the results of these series of experiments, one finds in the oxygen deficiency series lesions of two types, those of a progressive or hyperplastic variety, and those of a retrogressive or degenerative character, while in the carbon dioxide series degenerative lesions alone are noted.

Another interesting contrast in the two sets of experiments is shown in the location of the degenerative lesions within certain of the affected organs. Thus in the oxygen deficiency experiments the lesion increases in intensity with increasing distance from the arterial blood supply. On the other hand, in the carbon dioxide experiments

the lesions are in general more marked the nearer the tissues are to the arterial supply. These facts are especially clearly brought out in the liver and in the heart muscle. The difference seems due to the fact that in one case we are dealing with a deficiency of a vital element in the arterial blood supply, whereas in the other we are dealing with a toxic agent reaching the tissues through the same medium.

The hyperplasia of the bone marrow, that is extension of the red marrow throughout the cavities of the long bones, found in the oxygen deficiency series is the most marked example of the progressive lesion. This finding is in accordance with the results obtained by Dallwig, Kolls, and Loevenhart[2] in animals subjected to the same experimental procedure as that employed here. This is a direct stimulation of the cells of the marrow to proliferation and differentiation by decrease in oxygen fixation of these cells and forms with the more prompt response of the respiratory center a good example of an adaptive mechanism of the body. The occurrence of degenerative processes in these animals would indicate, however, that the compensation was not adequate for the extremely low oxygen percentage maintained in the experiments.

Whether the hyperplasia of the thyroid may be looked on as the result of a deficiency in oxygen or as the result of some uncontrolled factor in the maintenance of the animals before or during the experiment must, perhaps, remain unanswered. Slight hyperplasia was noted in the thyroids of animals subjected to an increased carbon dioxide pressure, though not nearly so marked as in the oxygen deficiency series. If the theory that the thyroid is the gland controlling internal oxidation is maintained, such a hyperplasia would be expected in oxygen deficiency. Conversely, the finding of hyperplasia of the thyroid under these conditions may be urged as evidence in favor of the theory. It is to be noted further that this hyperplasia as a result of oxygen deficiency is in agreement with the marked hyperplastic state of the human thyroid which may be found in pernicious anemia where there is also a marked oxygen deficiency. Since it is well known that increased thyroid activity increases oxidation in the body it may be assumed, we believe, that in this

[2] Dallwig, H. C., Kolls, A. C., and Loevenhart, A. S., *Am. J. Physiol.*, 1915–16, xxxix, 77.

hyperplasia of the thyroid we have another adaptation to oxygen want.

The interpretation of the more general degenerative lesion in the rabbits of the oxygen series offers somewhat greater difficulty. The lesion is of the nature of an increase in the size of the cell with an increase in its fluid content and with apparently a decrease in the protoplasm, a lesion which has been termed hydropic degeneration, vacuolar degeneration, or serous imbibition. This lesion cannot be attributed to the inability of the experimenters to control the food intake in animals in the cages. The histological picture is entirely different from the simple atrophy found in young rabbits accidentally starved as a result of their having eaten sawdust or shavings, which accumulated in the stomach. Moreover, as pointed out, the lesion in the various organs is most marked at those points which are farthest from the blood supply, where in extreme cases anemic necrosis was found. The lesion appears then to be due definitely to a deficiency in oxygen.

Confirmation of this was offered by the tissues of a dog in the laboratory stock which had extreme stenosis of the trachea due to a marked goiter. A similar hydropic lesion was found throughout the parenchymatous organs.

It is of interest in connection with the theory of thyroid function, cited above, that the lesions found in these animals and attributed to oxygen deficiency are identical with the lesions described by Tatum[3] in animals which had been thyroidectomized early in life. Tatum notes that in this experimental cretinism "degenerative changes have been noted in practically every parenchymatous organ. Among these the most striking has been that of serous imbibition by the most active cells of these organs." Accepting the theory as to the function of the thyroid, Tatum's results confirm the interpretation we have given our results. Again, the converse is true. Our results, clearly due to changes in but a single factor, that of oxygen supply, explain Tatum's findings and support the contention that the thyroid is concerned in vital oxidation.

An attempt has been made by us to explain the morphological changes resulting from reduced oxidation upon the basis of physico-

[3] Tatum, A. L., *J. Exp. Med.*, 1913, xvii, 636.

chemical changes within cells. On this basis one might assume that as a result of reduced oxidation there occurs an increased osmotic pressure within the cell and a consequent increased flow of water into the cells. On the other hand, one might assume that as a result of suboxidation, an acid condition of the cell occurs which would in turn lead to acid imbibition by the colloids of the cells according to the theory of Fischer. It should be borne in mind that while decreased oxidation probably causes increased acidity, there is evidence that increased acidity leads to decreased oxidation. The experiments which we have performed do not enable us to determine whether the swelling of the cells was due to acid imbibition of the colloids or to the osmotic factor. The lesion itself as seen in the liver cell, for example, in the oxygen series is identical in appearance with that seen in the rabbit in moderate chloroform poisoning, in ether intoxication, and in extreme fatigue, as well as in the condition where the carbon dioxide content of the air is maintained at a high level. In the case of chloroform poisoning Graham[4] has drawn the conclusion that the pathological changes in the cell are due to the liberation of hydrochloric acid through the decomposition of chloroform, although he states in addition that chloroform is "a drug which is known to suppress oxidations." In carbon dioxide poisoning one must assume a cell retention of carbon dioxide with increased acidity, autolysis, and probably also suboxidation. In fatigue it is assumed that the edema of the cell is due to accumulation of acid waste products.

CONCLUSION.

Exposure of rabbits to an atmosphere of low oxygen content results in a stimulation of the cardiorespiratory systems, in an extension (hyperplasia) of red bone marrow and probably of a thyroid hyperplasia, with the further production of hydropic and hyaline degeneration in the cells of the parenchymatous organs. An atmosphere of high carbon dioxide and normal oxygen content produces, however, a stimulation of the cardiorespiratory systems, but no marrow extension and, in the concentrations used, but slight hydropic degeneration in the parenchyma of the glandular organs.

[4] Graham, E. A., *J. Exp. Med.*, 1912, xv, 307; 1915, xxi, 185, xxii, 43.

EXPERIMENTAL ACUTE NEPHRITIS.

A Study of the Acidosis, Nitrogen and Chloride Retention, and of the Protective Action of Sodium Bicarbonate.

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In an earlier study (1) it was shown that experimental uranium nephritis in the dog is characterized by an acidosis and an increase in the blood urea and plasma chlorides and that when sodium bicarbonate is administered daily the acidosis is of lesser degree, the increase of blood urea and plasma chlorides is not so great, and the lesions in the kidney are not so severe.

In the present investigation the scope of the work has been extended to include other forms of nephritis, those due to cantharidin, arsenic, diphtheria toxin, and potassium chromate (2). Inasmuch as the general literature on the subject was reviewed and the methods of study were described in detail in the first paper, these will not be again considered.

The observations were made on the dog and include the determination of (*a*) the carbon dioxide content of the plasma by the Van Slyke-Stillman-Cullen method (3), (*b*) the non-protein nitrogen of the blood by the Folin-Denis method (4), (*c*) the urea nitrogen of the blood by the Van Slyke-Cullen method (5, 6), (*d*) the chlorides of the plasma by the McLean-Van Slyke method (7), (*e*) the ketone of the blood by the Rothera method (8), (*f*) the hydrogen ion concentration of the urine by the Palmer-Henderson method (9).

All the animals were kept on a constant diet for several days and normal control determinations made before the production of the nephritis. All the control observations of blood and urine were made on at least 2 different days. In order to ensure definitely comparable results all bleedings for the purpose of obtaining blood for

chemical study were made at 9 a.m. and the animals were always fed at 3 p.m. All the nephritic poisons were injected subcutaneously at 9.30 a.m.

The observations fall naturally into two groups: (*a*) the purely chemical and pathological studies of nephritis and (*b*) the study of the influence of sodium bicarbonate. For the first group a severe nephritis was produced and for the latter a lesion of milder degree. The study of a severe nephritis due to each of the four renal poisons will be described before the studies illustrating the influence of sodium bicarbonate are presented. The milder degree of nephritis will be described later in connection with the study of the effects of sodium bicarbonate.

Cantharidin Nephritis.

The animals received cantharidin (1 per cent) (Merck) dissolved in acetic ether in the proportion of 0.002 gm. per kilo of body weight. Albumin and casts appeared promptly in the urine. The general results of the chemical study—an increase in non-protein and urea nitrogen and in chlorides and a diminution in the carbon dioxide con-

TABLE I.

Cantharidin Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.*		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Mar. 1	6.5	30.3	14.3	5.2	59	7.3	
" 3	6.9	32.1	15.6	5.7	60	7.6	
Mar. 4	Injection of 0.002 gm. of cantharidin solution in acetic ether per kilo of body weight.						
Mar. 5	6.3	55.8	28.6	6.2	43	6.9	Vomits; polyuria; refuses food.
Mar. 6	Comatose; died at 12 a.m.						

* The carbon dioxide content was determined by the original Van Slyke method (3).

tent of the plasma—are shown in Table I. These results are duplicated in the second dog receiving the same dose of cantharidin. In the second dog the changes were in the same direction but not of the same degree. Histologically the kidneys of these animals showed marked congestion of the blood vessels, extreme granular degeneration of the convoluted tubules, and plugs of granular material in the tubular lumina.

Arsenic Nephritis.

In this series, arsenic in the proportion of 0.002 gm. per kilo of body weight was given.[1] The dose caused the appearance of albumin and casts in the urine. The chemical changes were in the same direction (Table II) as in the other forms of nephritis. In a second dog

TABLE II.

Arsenic Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Mar. 20	6.3	40.8	15.6	5.5	53	6.3	
" 22	6.2	39.2	18.2	6.0	55	5.9	
Mar. 23	Injection of 0.002 gm. of arsenious acid per kilo of body weight.						
Mar. 24	6.0	55.9	23.4	6.6	48	5.6	Comatose.
	Died the night of Mar. 24–25.						

which was given arsenic the changes were of even less degree, and a return to normal was seen in the second 24 hours. Histologically the changes in the kidneys were limited to granular degeneration of the epithelium of the convoluted tubules with accumulation of granular material in the lumina.

[1] Arsenious acid (Merck) was dissolved in weak sodium carbonate solution, the solution neutralized to phenolphthalein, and distilled water added to make a 1 per cent solution.

Diphtheria Toxin Nephritis.

In this series the animals received diphtheria toxin of approximately $\frac{1}{150}$ toxicity, in the proportion of 0.02 cc. per kilo of body weight. Albumin and casts appeared in the urine and after 2 days the chemical studies indicated most marked changes. These are shown in Table III. Like results, except that the chlorides were slightly lower, were obtained in a second animal similarly treated.

TABLE III.

Diphtheria Toxin Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Mar. 8	6.8	36.2	11.7	5.6	54	6.8	
" 10	6.8	36.6	15.6	5.9	53	6.7	
						6.6	
Mar. 14	10 a.m. Injection of 0.02 cc. of diphtheria toxin ($\frac{1}{150}$) per kilo of body weight.						
Mar. 15	6.4	41.2	20.8	5.8	50	6.2	Refuses food.
" 17	6.2	156.2	86.1	6.4	31		Comatose. 10 a.m. Died.

Histologically the kidneys of these animals showed intense congestion and extensive granular degeneration of the cells of the convoluted tubules with occasionally hyaline droplets and here and there necrotic cells. Casts, both granular and hyaline, were found in the tubules. The glomeruli were shrunken and the capsular spaces occasionally contained a finely granular material.

Potassium Chromate Nephritis.

Potassium chromate (Merck) was dissolved in distilled water to make a 1 per cent solution. The chromate salt was given subcutaneously, first in the proportion of 0.006 gm. per kilo of body weight and after 4 days a second injection of 0.01 gm. per kilo. Albumin and casts appeared promptly in the urine and death occurred, preceded

usually by vomiting and a comatose condition (Table IV). These results are duplicated in the second dog. Histologically the kidneys of these animals showed the typical degeneration and necrotic lesions of chromate nephritis.

The results of the cantharidin, arsenic, diphtheria toxin, and chromate nephritis confirm our earlier work with uranium nitrate and show that in experimental nephritis there occurs, in addition to

TABLE IV.

Chromate Nephritis.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Feb. 5	6.5	34.1	15.6	5.9	54	13.7	
" 6	6.4						
" 7	6.4	30.4	14.3	5.7	55	13.4	
Feb. 8	Injection of 0.006 gm. of potassium chromate (1 per cent solution) per kilo of body weight.						
Feb. 9	6.0	46.7	16.5	6.4	42	13.2	Refuses food.
" 11	6.3	88.6	28.2	6.1	46	13.1	" "
Feb. 13	Injection of 0.01 gm. of potassium chromate per kilo of body weight.						Refuses food.
Feb. 14	6.4	78.1	26.2	5.4	38	12.8	Refuses food.
" 15							Anuria.
" 16	6.5	118.5	41.6	5.7	36	11.7	Comatose. 10 a.m. Died.

the retention of non-protein and urea nitrogen, a moderate acidosis. This is most marked in the diphtheria and chromate nephritis, but is evident also to lesser degree in cantharidin and arsenic nephritis.

Effects of Sodium Bicarbonate.

In the series in which the protecting effect of sodium bicarbonate was studied, a milder grade of nephritis was produced. Four dogs

were used for each type of nephritis and the experiments were conducted so that two animals in each experiment received the bicarbonate, while the other two, serving as the controls, received none. To all the same dose per kilo of the nephritic poison was given. The animals receiving sodium bicarbonate were given the bicarbonate for several days before the nephritic poison was administered and the bicarbonate administration was continued throughout the experiment. The sodium bicarbonate was given by stomach tube in the proportion of 2 gm. per kilo of body weight with 10 cc. of water for every gm. of the salt. This daily treatment followed the drawing of blood at 9 a.m., which was also the hour at which the nephritic poison was administered. All food was given at 3 p.m.

TABLE V.

Cantharidin Nephritis. No Sodium Bicarbonate.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
May 10	6.8	34.0	12.3	6.0	53	6.3	
" 11	6.8						
" 12	6.8	39.3	15.2	6.0	53	6.2	
May 13	Injection of 0.0015 gm. of cantharidin per kilo of body weight.						
May 14	6.8	37.0	18.2	6.3	40	5.8	Vomits.
" 15	6.5						"
" 16	6.5	61.4	31.2	5.6	38		Does not eat; comatose.
May 17	Died at 1 p.m.						

Cantharidin Nephritis.—A comparison of Tables V and VI shows the influence of the sodium bicarbonate in preventing the reduction of the carbon dioxide content of the plasma. The animals receiving bicarbonate, moreover, showed less tendency to vomit and did not refuse food. Histologically the kidneys showed an equal degree of nephritis whether bicarbonate had been given or not.

TABLE VI.

Cantharidin Nephritis. Sodium Bicarbonate.

From May 8, 2 gm. of sodium bicarbonate per kilo of body weight were given after bleeding daily.

	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
May 8		36.8	13.0	5.6	54	5.9	Soda commenced after bleeding.
" 10	8.4						
" 11	8.7						
" 12	8.6	32.7	12.8	5.4	59	5.9	
" 13	8.6						
" 14	8.7						
" 15	8.7	30.3	12.4	6.1	59	5.9	
May 16	Injection of 0.0015 gm. of cantharidin per kilo of body weight.						
May 17	8.6	45.9	16.9	5.9	57	5.3	Vomits.
" 18	8.7						
" 19	8.6	36.2	12.8	6.3	54	5.3	
" 20	8.6						
" 21	8.6	35.6	10.4	6.5	61	5.1	Chloroformed.

TABLE VII.

Arsenic Nephritis. No Sodium Bicarbonate.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Apr. 21	6.5	30.6	14.8	6.0	52	7.3	
Apr. 23	6.7	31.6	9.1	6.0	52	7.3	
	Injection of 0.0015 gm. of arsenious acid per kilo of body weight.						
Apr. 24	6.0	33.3	13.7	6.7	44	7.1	
" 25	6.1						
" 26	6.2	40.8	18.7	6.6	45	7.1	Chloroformed.

Arsenic Nephritis.—In this series the difference between the control and the bicarbonate dogs was slight, but was in accord with that occurring in the preceding series. The dog that received bicarbonate showed no diminution of plasma carbon dioxide. The nephritis in all was of a very mild grade. None of the animals had disturbances of digestion and no difference in the degree of histological changes in the kidneys could be recognized.

TABLE VIII.

Arsenic Nephritis. Sodium Bicarbonate.

From May 3, 2 gm. of sodium bicarbonate per kilo of body weight were given after bleeding daily.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
May 3	6.7	32.8	10.4	6.0	52	8.6	Soda commenced after bleeding.
" 4	8.3						
" 5	8.3	35.6	14.3	6.0	58	8.5	
" 6	8.5						
" 7	8.5						
May 8	8.5	Injection of 0.0015 gm. of arsenious acid per kilo of body weight.					
May 9	8.7	35.3	15.6	6.4	55	8.3	
" 10	8.5						
" 11	8.3	33.2	19.5	6.3	55	8.2	Chloroformed.

Diphtheria Toxin Nephritis.—Tables IX and X show the same difference in carbon dioxide content as in the experiments with arsenic and cantharidin, but digestive disturbances occurred in both bicarbonate and control dogs. Histologically changes in the kidneys of the animals receiving bicarbonate were of slightly lesser grade than in those of the controls.

TABLE IX.

Diphtheria Toxin Nephritis. No Sodium Bicarbonate.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Apr. 6	6.9	38.2	11.7	5.8	52	5.9	
" 9	6.9	40.2	13.0	5.5	52	5.8	
" 11	6.9	35.9	13.0	6.0	54	5.6	
Apr. 12	Injection of 0.015 cc. of diphtheria toxin per kilo of body weight.						
Apr. 13	6.7	41.6	13.6	6.6	45	5.2	Vomits; refuses food.
" 14	6.7	83.3	46.8	6.2	36	5.1	" 4 p.m. Died.

TABLE X.

Diphtheria Toxin Nephritis. Sodium Bicarbonate.

From April 6, 2 gm. of sodium bicarbonate per kilo of body weight were given after bleeding daily.

Date.	Urine.	Blood.				Weight.	Remarks.
		Non-protein nitrogen per 100 cc.	Urea nitrogen per 100 cc.	Chlorides per liter.	CO_2 content in plasma.		
1917	*pH*	*mg.*	*mg.*	*gm.*	*vol. per cent*	*kg.*	
Apr. 6	6.8	36.4	10.4	5.6	51	5.8	Soda commenced after bleeding.
" 7	7.6						
" 8	7.8						
" 9	7.6	42.5	10.4	5.3	56	5.6	
" 11	7.8	39.3	9.1	5.8	60	5.5	
Apr. 12	Injection of 0.015 cc. of diphtheria toxin per kilo of body weight.						
Apr. 13	7.6	53.2	16.4	5.9	52	5.2	Vomits; does not eat.
" 14	7.6	62.2	46.8	6.2	53	5.0	"
	Died the night of Apr. 14–15.						

DISCUSSION.

The present work was undertaken to ascertain first what degree of acidosis occurs in experimental nephritis due to substances other than uranium, and second, how much protective action is obtained from sodium bicarbonate administered by stomach in these forms of nephritis It is clear that in the acute nephritis caused by cantharidin, arsenious acid, diphtheria toxin, and potassium chromate a moderate acidosis occurs, as shown by the diminution of the carbon dioxide content of the plasma and by the increased hydrogen ion concentration of the urine.

The non-protein and urea nitrogen and the chlorides in the blood, in the severe form, are always increased. In the milder grade of experimental nephritis, the action of the poisons was inconstant in the different dogs. While some dogs showed a moderate increase in the non-protein and urea nitrogen and in the chloride content of the blood; the others showed only very slight if any change. When sodium bicarbonate was given to these dogs the retention of nitrogen and of chlorides in the blood was in some instances less than in the control dogs but in other instances no difference could be noted.

In acute nephritis, a rise in the blood urea content may be due to renal insufficiency, and without doubt the impaired kidney function is to be considered the chief factor in the increase of the urea nitrogen in the blood, but such other factors as increased protein catabolism may be of some importance.

Gastrointestinal disturbances, indicated by the refusal of the animals to take food and by vomiting, frequently develop early in the course of the nephritis. Consequently the food intake of the nephritic dogs is not the same in different experiments and is not so constant as that during the preliminary control period. The possible influence of this change in food intake upon the catabolism must be considered, and it is not proper to attribute slight differences in the nitrogen content of the blood entirely to renal insufficiency.

Concerning the protective action of sodium bicarbonate against the acidosis it will be noted that the dogs receiving soda always showed a higher level of carbon dioxide content in the blood in the period before administration of the nephritic poison and, moreover,

that decrease in this figure during the nephritis was much less in the dogs receiving soda than in the controls without soda.

The hydrogen ion concentration was always diminished during the nephritic period in the control dogs, while in the dogs receiving soda it showed scarcely any change.

The most constant and striking difference between the control dogs and the dogs receiving soda in this mild grade of experimental nephritis was the less pronounced diminution of carbon dioxide content in the blood and the less pronounced decrease in the hydrogen ion concentration in urine in the dogs given soda, which indicates that the sodium bicarbonate has a protective action against the acidosis.

In some instances the animals when nephritic fell into a type of coma, being at first somnolent, then passing into a deep sleep, with deepened respiration. These symptoms are analogous to those seen in patients with uremic coma.

The association of severe acidosis and of coma is frequent. This suggested that there may be some relation between even the comparatively mild acidosis of these experiments and the greater tendency to coma that may be noted in the dogs receiving no soda.

Whether the acidosis of these types of experimental nephritis is due to a decrease of the excretory power of the kidney for acids, that is to retention of acids, or to the production of abnormal types of acids, or to increased production of acids is not known.

In all the animals acetone and acetone bodies were absent from the blood; hence, the acidosis in these types of experimental nephritis is not due to the acetone bodies.

CONCLUSIONS.

1. In experimental nephritis caused by cantharidin, arsenic, diphtheria toxin, and potassium chromate, in addition to the retention of the non-protein and urea nitrogen and of the chlorides in blood, an acidosis occurs.

2. Sodium bicarbonate given by the stomach has the power of diminishing the acidosis in these types of nephritis.

3. The degree of increase in the non-protein and urea nitrogen content in the blood in mild nephritis varies considerably in different dogs receiving the same dose per kilo of a given poison.

4. Histological examination shows little if any influence resulting from the administration of sodium bicarbonate upon the grade of the nephritis induced by the poisons.

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FURTHER STUDIES ON THE PROPERTIES OF PURE VACCINE VIRUS CULTIVATED IN VIVO.

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It has been shown that a sample of vaccinia virus, free from bacterial impurity and at the same time of sufficient strength for practical purposes, can be propagated in the testes of certain animals, particularly rabbits.[1] There were still several points, however, which seemed to require further investigation. It was not known whether or not, through continuous cultivation *in vivo* in the testicular tissues of rabbits, the biological properties of the virus would finally undergo modifications. Since the natural habitat of the virus is the skin, it is not improbable that the new medium in which it has been induced to grow might have a deleterious influence upon it. It was necessary to determine the viability of such a virus under varying conditions, that is at different temperatures, with the addition of antiseptics, etc. It was desirable also to compare the resistance and viability of the testicular strain with those of the virus propagated in the dermal tissue. These are questions not merely of purely academic interest, but of practical importance, inasmuch as the proper utilization of the new vaccine depends to a great extent upon the right understanding of its properties.

Scope and Mode of Experiments.

In the present series of experiments we studied the effect of disinfectants upon the testicular vaccine virus at different temperatures, the influence of diluents under various experimental conditions, the effects of desiccation upon the virus, and the viability of dried vaccine. The samples of testicular or of skin virus employed in each instance were highly active, being capable of producing a confluent eruption

[1] Noguchi, H., Pure cultivation *in vivo* of vaccine virus free from bacteria, *J. Exp. Med.*, 1915, xxi, 539.

on the shaved skin of the rabbit in 1: 1,000 dilution or higher. The vaccine preparations were placed in temperatures of 37°, 18°, 4°, and 0°C. Samples were taken from different test-tubes containing the virus at intervals in order to determine its strength under various conditions. During the 1st week the tests were made every 24 hours, since some of the vaccine samples which were kept at higher temperatures underwent a rapid attenuation of virulence, so that daily titration of their strength was imperative for following the course of deterioration.

The titration of the vaccine was made by applying a number of graduated concentrations of the specimen to a corresponding number of shaved areas, 10 by 20 cm., of the dorsal skin of rabbits. Special precautions were taken to have a control virus accompany the series of tests made on every animal in order to eliminate the irregular results due to individual variations in susceptibility to the vaccine virus, for without a proper control for each rabbit no accurate estimation of the vaccine effect may be made. In the present work duplicate tests were often resorted to. It was found both practical and reliable to make as many shaved areas as the dorsal and lateral surfaces of the rabbit's body permits, leaving narrow strips of hair between the shaved areas to serve as barriers, preventing the accidental overflow of one dilution to the next area. Readings of the results were made at intervals for about 8 to 10 days after inoculation. The dilutions of different samples varied according to the rapidity with which their strength diminished, but it was customary to prepare 1: 1,000, 1: 100, 1: 50, 1: 25, and 1: 10 dilutions, and undiluted stock suspension. The testing of different samples after the preliminary titration was, of course, much simplified, as in a subsequent titration fewer dilutions were sufficient to estimate the strength of the virus. Strict asepsis was exercised in handling the vaccine throughout the experiments.

For comparison the regular skin vaccines were employed. These had to be put up as emulsions containing 40 to 50 per cent glycerol or 0.5 per cent phenol.

Virulence and Affinities of the Testicular Vaccine Virus.

The first point to determine was whether a strain of vaccine virus which had been propagated for successive generations during a

period of 3 years would acquire an ascending virulence for the testicular tissues while suffering a gradual loss of its affinity for the tegumentary system. In the beginning the strain which was passed on to the testicular tissue showed a lower virulence for this organ, but it also showed a correspondingly low virulence for the dermal tissue. Upon attaining a certain degree of virulence for the testes the virus manifested a parallel increase of activity for the skin, showing no disproportion between the titers for the two kinds of tissue. It may be assumed, then, that by a prolonged passage through the testes, the affinity for the skin has in no wise been diminished. The maximum titer obtained by a testicular product in rabbits was that which produced a confluent eruption on the skin of rabbits in a dilution of 1:10,000. Such specimens were not frequently obtained, and the result probably depends upon the suitability of the animal used. There are considerable individual variations in the susceptibility of the rabbit and the calf, and it is not rare to get a specimen that requires a 1:100 dilution in order to produce a confluent reaction. The titers with the rabbits averaged about 1:1,000. Of course, a specimen possessing the activity to produce a confluent reaction in a 1:100 dilution is already strong enough to insure 100 per cent of takes among primates. The individual variations in suitability for producing a testicular virus are paralleled by the susceptibility of the skin of the same animal to the vaccine virus, regardless of the mode of propagation.

It has been stated elsewhere[1] that a highly potential testicular vaccine can easily set up vaccinia orchitis in the rabbit in dilutions as high as 1:100,000, while on the skin the same dilution produces a few eruptions. In this respect the virus seemed to have acquired an increase in virulence for the testicle but not necessarily to have lost its dermatophilic property. It still remained to be seen whether this orchitophilic adaptation of the virus was associated also with the general increase of virulence for other internal organs and tissues. In order to determine this point we tested the emulsions of lungs, liver, spleen, kidneys, and lymph glands, removed 5 days after inoculation, of the rabbits which had been successfully inoculated intratesticularly with the testicular virus. As controls a number of rabbits were intratesticularly inoculated with unadapted virus,

which, however, caused a marked orchitis. In a second series of animals the testicular strain was used on the skin, causing the latter to produce a confluent eruption, while several animals were vaccinated with the regular skin strain to serve as controls. The results were uniformly negative, except for a few eruptions in the areas inoculated with the lymph nodes in a few instances where the virus, irrespective of whether it was of testicular or dermal origin, was given intratesticularly.

Localization of the Vaccine Virus after Subcutaneous and Intravenous Inoculations.

The introduction of small quantities of the testicular virus, such as 1 cc. of a 1:1,000, 1:10,000, etc., dilution, into the blood circulation or under the skin of the rabbit produced no appreciable local or general symptoms. The injection of 1 cc. of a 1:100 dilution, however, sometimes caused a local inflammation and rise in temperature on the 4th and 5th days. No general eruption was ever observed. The injection of 1 cc. of a 1:10 dilution was sometimes accompanied by a high temperature for 3 days, and, in the case of subcutaneous injection, local tumefaction and edema, but no generalized eruption. The injection of 1 cc. or more of the undiluted emulsion produced a grave illness of 3 or 4 days, with a fever lasting for 3 days. Generalized eruptions, particularly numerous on the mucous membranes of the nose, lips, mouth, and genitalia, were observed. Camus[2,3,4] noted a similar phenomenon with the skin vaccine.

The examinations of different tissues removed from the animals showed that in cases of intravenous inoculation of 1:10 and 1:100 dilutions, the lymph glands and sometimes, but seldom, one of the testes contained some virus, but no bilateral orchitis or marked reaction was obtained. In case of a higher dilution than 1:1,000 we occasionally demonstrated a small quantity of the virus in the lymph

[2] Camus, L., De la vaccine généralisée expérimentale. Conditions de sa production, *Bull. Acad. méd.*, 1916, lxxvi, 342.

[3] Camus, L., Réproduction de la vaccine généralisée chez le chien, *Bull. Acad. méd.*, 1916, lxxvi, 376.

[4] Camus, L., La vaccine généralisée expérimentale chez la génisse et chez le singe, *Bull. Acad. méd.*, 1916, lxxvi, 433.

nodes, but never in the testes or other organs. Even the injection of 1 cc. of the undiluted vaccine failed in two experiments to localize in the testes. The other organs, except the lymph glands, were negative.

The corresponding series of experiments with subcutaneous inoculation did not bring about generalized eruptions even with the strongest dose used. A small amount of the virus in the adjacent lymph nodes was occasionally demonstrated, but far less frequently than in the intravenous series.

The rabbits which received the intravenous and subcutaneous inoculations of the virus were tested for their susceptibility to a subsequent application of a powerful vaccine, both the testicular and the dermal, within several weeks. They were found to be refractory to the new vaccination, although some of them had originally received only 1 cc. of a 1: 10,000 dilution. The testes of these rabbits were also insusceptible to the inoculation with a highly active virus. The immunity brought about by the intravenous or subcutaneous inoculation of the virus was altogether comparable with that conferred by a regular procedure on the skin. A detailed report of this phase of the work will be made later.

Viability and Resistance of the Testicular Vaccine Virus.

Data concerning the viability and resistance of vaccine virus ought to be abundant, but one does not easily find details of a systematic investigation. From the time of Jenner the resistance of the virus to desiccation has been known, and it was proved nearly 50 years ago[5] that it resists the action of glycerol when the latter is used in the proper concentration. Later Umeno[6] and others found that phenol in a concentration below 1 per cent does not perceptibly decrease its virulence. Yet much of what was done years ago seems to have been overlooked, and it may be of sufficient interest to publish here the experimental data obtained by the writer during the past 3

[5] Copeman, S. M., Vaccination. Its natural history and pathology, London, 1899, 156.

[6] Personal communication from Professor S. Umeno, Director of the Imperial Institute for the Preparation of Vaccine Virus, Tokyo, Japan.

years. Naturally, the chief object of the experiments was to study the testicular vaccine virus, but in some instances, controls with the regular vaccine virus were made as far as it was possible. From the nature of the latter, however, no experiments could have been carried out to test its viability in a suspension without antiseptics such as glycerol or phenol, while it was possible to do so with the bacteria-free testicular virus.

Survival of the Vaccine Virus in Distilled Water and Glycerolated Media, at Different Temperatures.

April 2, 1915. Three sets of eight test-tubes each were used. After sterilization, 11.6 cc. of distilled water were added to the first tube of each set, and to the other seven tubes 11.6 cc. of 10, 20, 40, 50, 60, 80 per cent, and pure glycerol respectively. The contents of each tube were mixed with 0.4 cc. of the stock emulsion of the testicular strain, No. 948 emulsion, which had the titer of 1: 1,000 (confluent). One set was placed in a refrigerator at 4°C., the second set at 18°, and the last at 37°C. 0.5 cc. was taken from each tube and tested as usual on the skin of rabbits, three or four rabbits being used in order to test the three sets (24 tubes) simultaneously. At first tests were made daily, or every other day, but later at semimonthly, monthly, or longer intervals. Tables I, II, and III give the results.

The most striking point demonstrated in the foregoing experiments is that the vaccine virus retains its virulence much longer in distilled water than in any of the glycerolated media. Pure glycerol destroyed the virus to a considerable extent in a week and completely within a month, even at a temperature of 4°C., while the virus in water remained very active after 1 year. As the concentration of glycerol diminished its destructive effect was less noticeable, and in 10 to 20 to 40 per cent the virus remained active for at least 6 months. At 18°C., the temperature of our laboratory, the virus deteriorated rapidly. The virus was killed in 5 days in pure glycerol, in 1 month in 60 to 80 per cent, in 2 months in 10 to 20 per cent glycerol, while it was still very active in water after 3 months. These differences are much more accentuated at 37°C., where the virus was no longer alive in pure glycerol after 24 hours. In 80 per cent glycerol it was avirulent in 6 days, in 60 per cent in 7 days, in 50 per cent in 9 days, and in 10 to 20 to 40 per cent in 28 days. On the other hand, in water

TABLE I.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 4°C.

April 9, 1915.

Medium.	Days.															
	1	2	3	4	5	6	7	9	11	13	15	28	43	60	173	271	360
Water										Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+++	++
10 per cent glycerol										"	"	"	"	"	"	+	<+
20 " " "										"	"	"	"	"	"	<+	−
40 " " "										"	"	"	"	"	"	<+	−
50 " " "										"	"	"	"	++	++	<+	−
60 " " "										"	"	"	"	++	+	−	
80 " " "	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	"	"	"	++	++	−		
100 " " "	"	"	"	"	"	"	+++	++	+	+	<+	−	−	−	−		

The reactions are designated as confluent (cfl.), almost confluent, +++, ++, +, <+, <<+, and finally, 1 to 3 eruptions to an area 10 by 20 cm., to which 0.5 cc. of each dilution was applied.

TABLE II.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 18°C.

April 9, 1915.

Medium.	Days.															
	1	2	3	4	5	6	7	9	11	13	15	28	43	60	173	271
Water										Cfl.	Cfl.	+++	+++	Cfl.	−	−
10 per cent glycerol										"	"	++	+	−	−	−
20 " " "										"	"	++	+	−	−	
40 " " "										"	"	+	<+	−		
50 " " "									Cfl.	"	+	<+	−	−		
60 " " "				Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	"	"	+	−	−	−		
80 " " "	Cfl.	Cfl.	Cfl.	"	+++	+++	+++	++	++	++	<+	−	−	−		
100 " " "	+++	++	+	<+	−	−	−	−	−	−	−					

TABLE III.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 37°C.

April 9, 1915.

Medium.	Days.													
	1	2	3	4	5	6	7	9	11	13	15	28	43	60
Water	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+++	++	<+	<+	<+	<+	< <+
10 per cent glycerol	"	"	"	"	"	"	"	++	+	<+	<+	−	−	−
20 " " "	"	"	"	"	"	"	++	+	+	+	<+	−	−	−
40 " " "	"	"	"	"	"	+++	++	+	+	+	<+	−	−	−
50 " " "	"	"	"	+++	++	+	+	−	−	−	−			
60 " " "	"	"	"	+	+	<+	−							
80 " " "	++	+	+	<+	<+	−	−							
100 " " "	−	−	−	−	−									

a trace of the virus was still present for as long as 2 months, when it still showed a few eruptions on test. The powerful vaccinicidal property of glycerol is well brought out in this set, and in any experiment bearing upon the viability of the virus this factor should be considered. The persistence of the virus in water or in 10 to 20 per cent glycerolated water at the temperature of 37°C. is remarkable and becomes important in interpreting the results in cultivation experiments.

Effect of Diluents upon the Viability of the Vaccine Virus at Different Temperatures.

May 24, 1915. Experiments were made similar to the foregoing but with distilled water, 0.9 per cent saline solution, Ringer's solution, 50 per cent glycerol, 0.5, 1, and 2 per cent aqueous, and 50 per cent glycerolated solutions of phenol as diluents. To 19.6 cc. of each of the solutions was added 0.4 cc. of testicular virus, No. 992 emulsion. The three sets of tubes were placed at 4°, 18°, and 37°C. respectively. The results of the tests are given in Table IV.

Table IV confirms the findings of the preceding series of experiments and further shows that at 4°C. the virus was best preserved in Ringer's solution and in 0.5 and 1 per cent phenol water, being still active at the end of 1 year. In distilled water it was weaker than in saline solution, the latter being almost as good a medium as Ringer's solution. The deteriorating effect of 50 per cent glycerol was marked in this instance. The addition of phenol in 0.5 per cent did not affect the action, although 1 per cent phenol plus 50 per cent glycerol killed the virus more quickly than either of them separately. Phenol in a 2 per cent solution and agitation of the virus with an excess of chloroform for 3 hours destroyed the virus within 24 hours. The results at 18°C. and at 37°C. indicate that there is a more complete and rapid destruction of the virus with a rise of temperature. An interesting feature seems to be the longer, if not better survival of the virus in a carbolized solution (0.5 per cent water) than in Ringer's solution, saline solution, or distilled water. In the first 7 days, the activity of the virus in the latter solutions was greater than in the carbolized medium, however.

TABLE IV.

Effect of Diluents upon the Viability of the Vaccine Virus at Different Temperatures. 19.6 Cc. of Diluent + 0.4 Cc. of No. 992 Emulsion (1: 50 Dilution).

May 24, 1915.

Temperature.	No. of days.	Water.	0.9 per cent saline solution.	Ringer's solution.	50 per cent glycerol.	Phenol in water.			Phenol in 50 per cent glycerol.			Shaken 3 hrs. in aqueous suspension with.	
						0.5 per cent.	1 per cent.	2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	Chloroform.	Ether.
°C.													
	1	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	−	Cfl.	+++	−	−	+
	2	"	"	"	"	"	"	−	"	+++	−	−	+
	3	"	"	"	"	"	"	−	"	+++	−	−	+
	4	"	"	"	+++	"	"		"	+++		−	+
	5	"	"	"	+++	"	"		"	++		−	+
4	7	"	"	"	++	"	"		++	+			−
	17	"	"	"	+	"	"		+	<+			
	37	"	"	"	+	"	"		+	<+			
	226	+++	"	"	−	"	"		<+	<+			
	276	<+	+++	"	−	"	"		−	−			
	365	++	+++	"		"	"						
	1	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	−	+++	+++	−	−	<+
	2	"	"	"	"	"	+++	−	+++	+++	−	−	< <+
	3	"	"	"	"	"	++	−	++	++	−	−	−
	4	"	"	"	"	"	++		++	+			−
	5	"	"	"	<+	"	+		<+	<+			
18	7	"	"	"	1 eruption.	"	+		−	−			
	17	+	"	+	−	+	<+		−	−			
	37	+	+	+	−	+	−		−				
	173	−	−	−	−	−	−		−				

| | | |

Survival of the Vaccine Virus in Different Atmospheres.

In order to find whether a gradual deterioration of the vaccine virus could be delayed or prevented by preserving the virus in different kinds of gases, we placed the vaccine in sealed ampules containing either hydrogen, nitrogen, oxygen, carbon dioxide, or air. For this purpose the testicular vaccine, No. 1,035 emulsion (1: 1,000 titer), was diluted 10 times with sterile distilled water, the gases were passed through the vaccine, and the receptacles hermetically sealed by fusing the drawn portions with flame. The vaccine was not exposed to heat.

The appearance of the vaccine was not altered by hydrogen, nitrogen, or air, but the passage of carbon dioxide caused a clearing of the diffuse turbidity, the precipitates adhering to the wall of the container, while with oxygen, granular precipitates appeared.

Three duplicate sets placed at 4°, 18°, and 37°C. respectively were used. The results obtained after a period of 21 days are shown in Table V.

TABLE V.

Survival of Vaccine Virus in Various Atmospheres.

Gas.	Results after 21 days.		
	4° C.	18° C.	37° C.
Hydrogen (sealed)	Cfl.	+++	–
Nitrogen "	"	++	–
Oxygen "	"	–	–
Carbon dioxide (sealed)	"	–	–
Air (sealed)	"	++	–
" (open tube)	"	+	–

The virus retained its virulence in all the gases for 3 weeks when kept at 4°C. but became avirulent at 37°C. From the results obtained at 18°C., however, it may be concluded that in sealed ampules containing hydrogen, nitrogen, or ordinary air, the virus retained its virulence somewhat better than in an open receptacle. Pure oxygen or carbon dioxide gas destroyed the virus completely at the same temperature.

Effect of Acid, Alkali, and Germicides upon the Vaccine Virus.

Knowledge concerning the resistance of the vaccine virus to various substances is a prerequisite for handling it properly. In a series of experiments hydrochloric acid, sodium hydroxide, tricresol, and phenol in varying concentrations were mixed with a testicular vaccine emulsion. Tests were made on rabbits at the end of 24 hours, 4 days, and 6 days respectively at 4°C. Table VI shows the results.

The table shows that the vaccine virus is completely destroyed by sodium hydroxide added in a concentration greater than 1: 200,

TABLE VI.

Effect of Acid, Alkali, and Germicides.

Substance.		Testicular vaccine (no bacteria). Tested for vaccine.			Fresh skin vaccine (some cocci).					
					Tested for vaccine.			Tested for bacteria.		
		24 hrs.	4 days.	6 days.	24 hrs.	4 days.	6 days.	24 hrs.	4 days.	6 days.
	per cent									
Hydrochloric acid.	4	−	−	−	−	−	−	−		
	2	−	−	−	−	−	−	−		
	1	−	−	−	−	−	−	−		
	0.5	1 eruption.	−	−	A few eruptions.	−	−	−		
Sodium hydroxide.	4	−	−	−	−	−	−	−	−	
	2	−	−	−	−	−	−	−	−	
	1	−	−	−	−	−	−	+	−	
	0.5	−	−	−	−	−	−	+	−	
Tricresol.	2	−	−	−	−	−	−	−	−	−
	1	−	−	−	−	−	−	−	−	−
	0.5	Cfl.	−	−	Cfl.	−	−	−	−	−
	0.2	"	Cfl.	Cfl.	"	Cfl.	Cfl.	+	+	+
Phenol.	2	−	−	−	−	−	−	−	−	−
	1	Cfl.	Cfl.	+++	Cfl.	Cfl.	+++	+	−	−
	0.5	"	"	Cfl.	"	"	+++	+	+	+
	0.2	"	"	"	"	"	+++	+	+	+
Control.		Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+	+	+

while hydrochloric acid destroyed it almost completely in a corresponding concentration. On the other hand, the contaminating micrococci of the fresh skin pulp showed a different relation, resisting the action of even 1 per cent sodium hydroxide solution for at least 24 hours, while they were completely sterilized by a 0.5 per cent hydrochloric acid solution. The action of tricresol and phenol is similar, the difference being quantitative rather than qualitative. The virus resisted 0.2 per cent tricresol or 0.5 per cent phenol for many days, as did also the contaminating bacteria. In the case of phenol, and in a lesser degree tricresol, the destructive effect was comparatively more severe upon the micrococci than upon the vaccine virus. The margin was, however, narrow. A bacterial spore cannot be sterilized by a concentration which will not destroy the vaccine virus completely.

The action of iodine was next studied in various ways, because of its effectiveness as a germicide. It was employed as a local antiseptic in the form of an alcoholic solution or as Lugol's solution. In a series of experiments we made a number of dilutions of tincture of iodine by using 10 per cent ethyl alcohol water as diluent. To 0.9 cc. of each dilution, 0.1 cc. of a 1: 10 dilution of a strong testicular vaccine virus was added, the mixture incubated for 1 hour at 37°C., and then tested on rabbits. It was found that the diluent alone, that is a 10 per cent alcohol water, exerted no effect. On the other hand, the mixtures containing a dilution of the tincture of iodine above 1: 10,000 became avirulent. The mixture which contained a dilution of 1: 100,000 gave several eruptions instead of the confluent reaction which took place when controls without iodine were used. The experiment demonstrates how destructive this element is for the vaccine virus. Lugol's solution (iodine 1 part, potassium iodide 2 parts, and water 300 parts) destroyed the virus in a 1: 100 dilution but had no effect in a 1: 1,000 dilution. Attempts were made to influence the course of the vaccine reaction by administering a considerable amount of Lugol's solution or sodium or potassium salts by intravenous or subcutaneous injections for several days before and after the vaccination. No effect was perceptible. The iodide salts failed to reduce the virulence of the vaccine virus even when mixed *in vitro* in a 30 per cent solution and kept 1 hour at 37°C.

Attempts were made to sterilize the vaccine virus simultaneously

with its application to the skin or at various intervals afterwards. It was found that tincture of iodine in a concentration stronger than 1:10 inhibits the development of the eruption; in 1:400 dilution, it prevented the process from being confluent. Lugol's solution used in full strength reduced but failed to check the infection. The application after 24 hours of tincture of iodine in concentrated forms did not noticeably influence the vaccine infection.

So far no visible organism has been found as the causative agent of vaccinia. From the viewpoint of classification it seemed important to study the effects of certain protoplasmic poisons on the virus. For this purpose 0.1 cc. of testicular vaccine, No. 1,062 emulsion, was mixed with 0.5 cc. of sodium oleate, sodium taurocholate, sodium glycocholate, and sodium cholate, in varying concentrations. After 1 hour at 37°C. the mixtures were tested on rabbits. It was found that all the salts destroyed the vaccine virus in a 1:10 dilution. In a 1:100 dilution a small portion of the virus still survived, while the virus in control tubes was capable of producing a confluent reaction.

Effect of Desiccation upon the Vaccine Virus.

Vaccine virus is known to withstand desiccation for a long time, but more exact knowledge is desired as to its reaction to dryness, the length of time it will survive in the dry state, and how the dried vaccine virus compares with moist emulsions at different temperatures. Many microorganisms, especially those which pass the filter, resist desiccation and remain viable for a long time. Most enzymes retain their activity better in the dry than in the moist state, especially at the higher temperatures. The question becomes one of considerable importance in the case of the vaccine virus, because of the rapid deterioration which attends the moist preparation of the virus in tropical countries. If the dried vaccine proved to resist the conditions of moisture and temperature similar to those of a tropical climate better than the liquid emulsion, it would be a great advantage to preserve the vaccine virus in the dried form.

On several occasions we have dried quantities of the organ paste of testicular vaccine virus by means of a vacuum pump. After desiccation quantities were weighed, powdered, and preserved in

hermetically sealed ampules or left in an open receptacle. One set of specimens was placed at 4°C., some at 18°, and others at 37°. The controls consisted of aqueous emulsions of the undried portion of the same tissue paste.

The reduction in weight through desiccation was not uniform, and no constancy could be expected on account of the variation in degree of edema of the organs (Table VII).

TABLE VII.

Reduction of the Weight of the Vaccine Paste through Desiccation.

Emulsion No.	Organ.	Original weight.	Residue.
		gm.	*gm.*
866	Testis.	2.0	0.38
876	"	2.5	0.38
877	"	2.0	0.3
878	"	2.0	0.32
1,045	"	1.85	0.32
1,046	"	1.6	0.25

The vaccine virus did not in any instance show its original titer after desiccation. The loss of virulence, as determined by employing corresponding quantities of the dried and moist materials, amounts to half or even more of its original strength. This was unexpected, but was true with all the dried materials. The insolubility which attends the desiccation of the vaccine paste may play a part in the loss of virulence. To resuspend and dissolve the powdered material is difficult. The results obtained during a period of 18 months indicate that the dried material remains still viable, although reduced to one one-hundredth or less of the original titers, for about 12 to 18 months at 4°C. and 18°C., but becomes inert within 30 to 60 days at 37°C. The control specimens in the moist state showed similar relations.

From the above findings it is evident that the process of desiccation as carried out is considerably destructive to the vaccine virus, and that it does not protect it from the gradual deterioration due to age which takes place at different temperatures.

SUMMARY.

1. The virulence of vaccine virus for the testicular tissues increases until its maximum is finally reached. The selective increase is not associated with any loss, reduction, or modification in its virulence for the skin. A highly potent testicular vaccine is also highly active for the skin.

2. The testicular strain of vaccine virus has no more tendency to localize in various organs than the ordinary skin strain. Both may localize in adjacent lymph nodes when introduced intravenously, subcutaneously, or intratesticularly in sufficiently large quantities, but other organs are not involved.

3. Intravenous inoculation of an excessive amount of a powerful vaccine virus (1 to 2 cc. of undiluted stock emulsion), irrespective of whether it is from the testis or the skin, will result in a generalized eruption over the entire body surface of rabbits. The eruption may be confluent on mucous membranes of the mouth, nostrils, genitalia, etc. Intratesticular or subcutaneous inoculations of the same virus fail to produce this effect.

4. Subcutaneous or intravenous introduction of much smaller quantities of the virus does not cause an appreciable local or general reaction in the rabbit. But the animals which have once received these injections become refractory to a subsequent vaccination as applied to the skin. It seems probable that an active immunity has been conferred.

5. Experiments on the viability and resistance of the testicular strain of vaccine virus indicate that the virus is best preserved when emulsified with Ringer's solution or 0.9 per cent saline solution. Distilled water, while apparently one of the best diluents, fails to keep the virus active as long as Ringer's or saline solutions. As would be expected, the lower the temperature is, the longer the virus retains its viability. At 18° or 37°C., the deterioration of the virus proceeds rapidly. However, a small part of the virus survives after many weeks' standing at 37°C.

6. Of the two most commonly employed chemical agents for the ripening (eliminating bacteria) process of the green vaccine pulp, glycerol and phenol, the latter is the less injurious. Phenol in con-

centration above 2 per cent destroys the virus within 24 hours at any temperature, but it has almost no injurious effect when used in 0.5 to 1 per cent. On the other hand, glycerol is a powerful vaccinicide. When used in full strength it destroys the virus within 24 hours, even at 4°C. In a concentration of 40 per cent, that ordinarily recommended for the ripening, the virus retains some of its virulence for about half a year at 4°C., while at higher temperatures the same concentration kills the virus within 1 to 2 months. The virus preserved in distilled water or Ringer's solution under similar temperature conditions remains more active during this period. From this it may be concluded that glycerol is not an indifferent agent, as is assumed by many, but a powerful vaccinicide when used in high concentrations. The injurious effect is markedly accelerated at 18° or 37°C.

7. The vaccine virus retains its virulence better in a sealed tube containing either hydrogen, nitrogen, or air than in an open receptacle. The virus deteriorates when placed in a sealed tube with oxygen or carbon dioxide.

8. Desiccation decreases to a considerable degree the virulence of the vaccine virus. In the dried state the virus retains its viability about as long as does the emulsion, but it is not protected from the deterioration caused by age under various conditions.

9. Iodine is a powerful disinfectant for the vaccine virus, but its sodium and potassium salts have no effect. Various bile salts destroy the vaccine virus when employed in sufficient concentration.

SURVIVAL OF POLIOMYELITIC VIRUS IN THE BRAIN OF THE RABBIT.

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The consensus of opinion among investigators in America and Europe is that the causative microorganism of poliomyelitis is a so called filter passer. The filterable organism or virus of poliomyelitis possesses definite properties through which it may be identified, and among these the most decisive is its ability to incite experimental poliomyelitis in the monkey. In common with other filter passers, the microbic cause of poliomyelitis is very minute. It has not been detected with certainty by microscopic examination in infectious filtered fluids. There are reasons for believing that in artificial cultures the microorganism gives rise to colonies visible to the naked eye and composed of masses of minute organisms which have been called globoid bodies.[1] Similar globoid bodies have been detected in microscopic preparations of the nervous organs[2,3] and once in the circulating blood of an infected monkey.[3]

Another point of view, emphasized more recently, is based on the studies of Mathers, Rosenow, Nuzum, and their associates.[4] According to these investigators, epidemic poliomyelitis is caused by a polymorphous streptococcus, which induces paralysis and histological changes characteristic of the disease in rabbits as well as in monkeys.

[1] Flexner, S., and Noguchi, H., *J. Am. Med. Assn.*, 1913, lx, 362.

[2] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

[3] Amoss, H. L., *J. Exp. Med.*, 1914, xlx, 212.

[4] Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx, 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxvii, 1202; *Science*, 1916, xliv, 614; *J. Am. Med. Assn.*, 1917, lxviii, 280. Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205. Nuzum, J. W., *ibid.*, 1916, lxvii, 1437; 1917, lxviii, 24.

Since the filtered virus of poliomyelitis, while highly active against monkeys, is practically without pathogenic power for rabbits, a wide difference of experimental results needs to be explained in order to bring the two points of view into harmony.

Bull,[5] in this laboratory, attempted to confirm the work referred to with the polymorphous streptococcus, but without success. He carried out a large series of inoculations of streptococci derived from poliomyelitic human and monkey tissues without, in a single instance, either in rabbits or monkeys, inducing clinical symptoms or pathological lesions identifiable with those of epidemic poliomyelitis. Moreover, his efforts to immunize animals with the streptococcus so as to obtain a neutralizing serum for or to protect them against infection with the filtered virus, as Rosenow claims to have done, were wholly unsuccessful. More recently, Bull has again tried, unsuccessfully, to render a monkey immune to the virus by large intravenous injections of streptococci cultivated from the brain of a poliomyelitic human case. The protocols of this experiment follow. The question, therefore, arises as to the source as well as the significance of the streptococci found not infrequently in poliomyelitis. Smillie[6] found that when the cultures are made from monkeys moribund and slowly dying, or from animals which have been dead some hours, streptococci are frequently present, not only in the nervous organs, but even more abundantly in the abdominal viscera. In other words, the streptococci exhibit the characters of secondary, agonal invading microorganisms. The unreported experiment of Bull with streptococci follows. *Macacus rhesus* monkeys were used.

Monkey A.—Apr. 25, 1917. Injected intravenously the centrifuged sediment from a 24 hour growth in 60 cc. of ascitic dextrose broth of the third generation of streptococcus obtained from human poliomyelitic brain. The monkey remained active. Apr. 27. Injected intravenously the growth from 56 cc. of the same medium, third generation of the same streptococcus, and intracerebrally the growth from 14 cc. of the same culture. The monkey became irritable, but remained active, and was normal on May 1 when another intravenous injection from 60 cc. of the third generation culture was made. May 5. A fourth injection was given.

This monkey, the serum of which was shown on May 22 to agglutinate the strain of streptococcus in a dilution of 1:4,000, was tested (*a*) for neutralizing action on the filtered poliomyelitic virus, and (*b*) for protection against an intracerebral inoculation of the same virus.

Monkey B. Neutralization Test.—May 22. 2 cc. of serum from Monkey A were mixed with 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of poliomyelitic monkey cord (active virus), incubated for 2 hours at 37°C., and placed

[5] Bull, C. G., *J. Exp. Med.*, 1917, xxv, 557.

[6] Smillie, W. G., *J. Exp. Med.*, 1917, xxvii, 319.

over night at 4°C., then injected intracerebrally into Monkey B. May 30. Animal excited. June 1. Ataxic. June 2. Legs paralyzed; back weak. June 3. Prostrate. June 4. Died.

Autopsy.—Typical gross and microscopic lesions of poliomyelitis were present in the central nervous organs.

Protection Test of Monkey A.—May 22. After blood was withdrawn for the serum an intracerebral injection of 0.5 cc. of active virus was made. May 27. Monkey excited and ataxic. May 28. Prostrate. May 29. Died.

Autopsy.—Typical poliomyelitic lesions were present in the central nervous system.

EXPERIMENTAL.

In order to study further the relation of the filterable virus of poliomyelitis to the rabbit, with the special view of bringing out resemblances to or distinctions from the streptococcus and of determining its power of survival in the brain *in vivo*, inoculations of the virus were made into the brain of that animal. There was no expectation of inducing infection or of setting up paralysis. Bull[7] injected streptococci isolated from the human poliomyelitic brain intravenously into a rabbit without producing symptoms. The rabbit was etherized after 131 days. Streptococci corresponding antigenically with the strain originally injected were found to have survived in the brain. Bull also observed that local injections of streptococci from poliomyelitic tissue sometimes produce focal lesions in which the organisms survive for long periods. In other words, the polymorphous streptococcus is, under certain conditions, sufficiently adapted to the central nervous system of the rabbit to survive there, and sometimes sufficiently pathogenic to produce focal lesions in the meninges, cerebellum, medulla, cerebrum, and even in the spinal cord, and to thus induce clinical symptoms. The lesions do not, however, partake of the nature of the characteristic lesions of poliomyelitis.[5]

Hence, if a relation exists between the polymorphous streptococcus and the filterable virus, the latter might at least be expected to exhibit a fair degree of ability to survive in the brain of the rabbit. As the protocols which follow show, the period of survival is short.

[7] Unpublished experiment.

Rabbit A.—Nov. 21, 1916. Under ether anesthesia 0.5 cc. of a suspension of equal parts of active poliomyelitic monkey cord and isotonic salt solution was injected intracerebrally. The rabbit remained well. Dec. 14. The animal was etherized and the brain removed aseptically. There was no visible lesion at the site of inoculation. A 10 per cent suspension of the brain tissue from the region below the point of needle penetration through the skull was prepared for injection into a *Macacus rhesus* (Monkey C).

Monkey C.—Dec. 14, 1916. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit A, which had received the poliomyelitic virus 22 days previously. The monkey remained well.

Rabbit B.—Jan. 3, 1917. Injected heavy suspension of poliomyelitic virus according to the method already described. Jan. 16. Killed. There was no visible lesion at the site of inoculation. From the brain substance surrounding the site of inoculation, a 10 per cent suspension was prepared for injection into a *Macacus rhesus* (Monkey D).

Monkey D.—Jan. 16, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of brain at site of inoculation of Rabbit B, which had received poliomyelitic virus 12⅔ days previously. The monkey remained well.

Rabbit C.—Mar. 5, 1917. Injected intracerebrally heavy suspension of poliomyelitic virus according to the method already described. The rabbit showed no symptoms. Mar. 12. Killed. There was no visible lesion at the site of inoculation. The brain was removed aseptically and a 10 per cent suspension of the brain substance around the site of inoculation was prepared for injection into a *Macacus rhesus* (Monkey E).

Monkey E.—Mar. 12, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit C, which had received poliomyelitic virus 7 days previously. The monkey remained well.

Rabbit D.—Apr. 3, 1917. Injected intracerebrally a heavy suspension of active poliomyelitic monkey cord. The rabbit remained well. Apr. 7. Etherized and brain removed aseptically. No visible lesion at site of inoculation. A 10 per cent suspension was prepared from the brain tissue at the site of inoculation and injected intracerebrally into a *Macacus rhesus* (Monkey F).

Monkey F.—Apr. 7, 1917. Injected intracerebrally 2 cc. of the 10 per cent suspension of the brain at the site of inoculation of Rabbit D, which had received poliomyelitic virus 4 days previously. Apr. 13. Both legs paralyzed. Apr. 14. Prostrate. Apr. 15. Died.

Autopsy.—The central nervous organs showed macroscopic and microscopic lesions of poliomyelitis.

DISCUSSION AND SUMMARY.

Suspensions of the central nervous tissues of monkeys, containing the active filterable virus of poliomyelitis, may be injected into the

brain of rabbits without setting up symptoms, provided the volume of injection does not cause dangerous increased intracranial pressure.

Aside from the pressure effects which develop quickly, no other symptoms or pathological lesions are produced by the suspensions.

The active virus of poliomyelitis survives in the brain of rabbits for 4 days, as determined by tests in the monkey, into which the excised site of injection in the rabbit brain is reinoculated. It cannot be detected by this test after the expiration of 7 days.

The virus of poliomyelitis is unadapted to the rabbit, and neither induces lesions nor survives long in the central nervous organs of that animal. In this respect it differs from certain streptococci cultivated from poliomyelitic tissues.

A monkey immunized to streptococcus cultivated from human poliomyelitic nervous tissues yielded a serum which agglutinated the streptococcus in high dilution, but was without neutralizing action on the filtered virus; and the streptococcus-immune monkey was not protected against the effects of an intracerebral inoculation of the filtered virus.

The experiments recorded provide additional reasons for concluding that the streptococcus cultivated from cases of poliomyelitis differs essentially from the filterable virus and is not the microbic cause of epidemic poliomyelitis.